HEPATITIS C AND G IN SOUTH EAST SCOTLAND STUDIES IN EPIDEMIOLOGY, PROGRESSION OF DISEASE AND QUALITY OF LIFE

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A thesis submitted for Doctor of Medicine (MD) to the University of Edinburgh

October 2001

Declaration

I declare that this thesis is exclusively of my own composition and is based on the results of my own work, except where stated otherwise. The research was carried out whilst I was a research fellow in the Department of Medicine. The data presented in this thesis have not been submitted previously for a higher degree.

MBChB (Edinburgh), MRCP (UK)

October 2001

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Acknowledgements

I would particularly like to thank Professor Peter Hayes for all his guidance, support and encouragement throughout the period of my research and writing up of my thesis.

I am grateful to Professor Peter Simmonds, Fiona Davidson, Carol Lycett and Deborah Macdonald who performed all the virological techniques in the hepatitis G study and who collaborated in the setting up of the study.

I am also grateful to Dr J Gillon and the staff in SNBTS for their help and support with the hepatitis G study.

I thank Mary McColl and Lorraine Dean who performed the majority of the HLA typing in the study and Dr PL Yap for putting up with me during the HLA study.

I also thank Sister Margo Miller, Sister Ruth Miller, Maggie Couston and Professor Ronan O'Carroll for their help and advice with the quality of life study.

I am indebted to Geoff Haydon for all his advice and enthusiasm throughout the period of my research. Also thanks to Toby Delahooke.

I would like to thank my parents for all their interest and support over the years and finally Dave, Mairi, Isla and Alasdair for their encouragement and patience.

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ABBREVIATIONS USED

AICAH	Autoimmune chronic active hepatitis
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CTL	Cytotoxic T lymphocyte
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immunosorbent assay
HAD	Hospital anxiety and depression scale
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HGV	Hepatitis G virus
HIV	Human Immunodeficiency Virus
HLA	Human Leucocyte Antigen
HRQOL	Health related quality of life
HVR	Hypervariable region
IDDM	Insulin dependent diabetes mellitus
IRES	Internal ribosomal entry system
IVDU	Intravenous drug user
MHC	major histocompatability complex
NANB	non-A, non-B hepatitis
NSAID	non-steroidal anti-inflammatory drug
OLT	Orthotopic liver transplantation
ORF	Open reading frame
PBC	Primary biliary cirrhosis
PCR	Polymerase chain reaction
PSC	Primary sclerosing cholangitis

PTH	Post transfusion hepatitis
QOL	Quality of Life
RIBA	Random immunoblot assay
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase polymerase chain reaction
SF-36	Short form 36
SIP	Sickness impact profile
STD	Sexually transmitted disease
TAH	Transfusion associated hepatitis
UTR	Untranslated region
WHO-QOL	World Health Organisation Quality of Life Questionnaire

ABSTRACT

Hepatitis C is now a global health problem with an estimated 170 million people infected worldwide. It is known to be the cause of the majority of cases of non-A, non-B hepatitis. It is known to be progressive with at least 20% of patients developing cirrhosis over 20 years. What is less clear is whether all infected people with eventually develop complications and whether certain factors are important in progression of this disease. There are other similar viruses that have been characterised including HGV and their importance is not yet entirely clear.

The aims of this thesis were to:

Study the epidemiology and natural history of HCV infection in our population of HCV infected patients in South East Scotland.

Investigate the factors important in progression of chronic HCV infection and also the effects of chronic HCV infection on quality of life

Examine another novel flavivirus, HGV, in Scottish blood donors.

A database of all patients with chronic HCV referred to our hospital was set up. Data was gathered prospectively and analysed with regards to demographic variables and factors related to progression of disease. The importance of HLA status in development and severity of disease was also examined. Finally the impact of chronic HCV infection on QOL was assessed in a subgroup of this population.

In a separate population the prevalence, natural history, clinical significance and risk factors for transmission of HGV were assessed.

262 patients were initially included in the database. 214 patients were HCV-RNA positive and were investigated further. Various demographic variables were recorded. Nearly 20% had cirrhosis on biopsy. Factors important in progression were age at infection, male sex and alcohol consumption.

When HLA status and progression were examined certain antigens were found to protect against development of chronic disease and cirrhosis.

Quality of life was found to be significantly impaired across all domains of life in patients with chronic HCV infection.

HGV is common amongst Scottish blood donors but is not associated with significant symptoms or liver disease. A risk factor for infection was not identified in the majority of infected donors.

CHAPTER ONE

INTRODUCTION

1.1 Introduction

Hepatitis C virus infection (HCV) is now known to be responsible for 90% of the cases of non-A, non-B hepatitis and nearly all those that followed blood transfusion. It is the most common cause of liver disease in the United States and is estimated to have infected 170 million people worldwide. It is now the most common indication for OLT.

The virus was first cloned in 1989 (Choo et al 1989) and since that time considerable progress has been made in characterising the structure and biology of the virus, identifying the mechanism of hepatocellular damage, recognising the routes of transmission and the development of sensitive and accurate diagnostic tests.

1.2 Prevalence

The prevalence of infection varies throughout the world. In the UK the prevalence is estimated at 0.075%-1.5%, at 0.3% in North America and Western Europe, 0.5%-1.5% in the Mediterranean and Eastern Europe and 2% in South America (Alter 1995). In Africa the figure is reported as between 0.6% and 1.5% and in Egypt up to 20%. In the East the prevalence varies from <1% in Australia, New Zealand and China, to >2% in Japan and Saudi Arabia.

1.3 Transmission

The main route of transmission is parenteral. The highest incidence occurs in intravenous drug users (IVDU) and recipients of blood and blood products prior to implementation of the screening regimens for blood donors.

Blood Transfusion

Prior to the introduction of HCV antibody testing in 1990 estimates of the frequency of transfusion associated NANB hepatitis were available. These range from 0.5% in UK to 3-4% in USA, 7.7% in Japan, 9.6% in Spain and 12.5% in Taiwan. Since the implementation of blood donor screening with anti HCV the risk of post transfusion hepatitis has significantly declined. The current estimated risk is between 0.05%-0.017% per unit of blood. (Donahue et al 1992; Kleinman et al 1992). With the routine introduction of PCR testing the risk will further decline.

Patients who require repeated blood transfusions are at much higher risk of contracting HCV infection. (Mozzi et al 1991, Locasciulli et al 1991).

Plasma Product recipients

Unfortunately a high proportion of haemophiliacs became infected with the virus prior to the introduction of current methods of inactivation in pooled plasma products and blood donor screening programmes. Haemophilia patients who have received repeated transfusions with untreated or incompletely activated factor concentrates have prevalence rates of HCV in excess of 90%. (Rumi et al 1990; Troisi et al 1993).

Intravenous drug users.

As a result of needle sharing amongst IVDU the prevalence of HCV infection is significant. In one Spanish group 86% were positive for HCV antibodies and of those with less than one year of intravenous drug use the prevalence rate was 69%. (Bolumar et al 1996). IVDU who have a longer duration of use, a history of needle sharing and a history of imprisonment are at the greatest risk of infection. (Crofts et al 1993;Donahue et al 1991)

Haemodialysis patients

The prevalence in haemodialysis patients varies according to geographical area. In the UK we have one of the lowest prevalence rates compared to a rate of 20-91% in Eastern Europe. (Alter 1995). Several studies have shown an association between increasing years on dialysis and rates of HCV antibody positivity. (Niu et al 1993; Hardy et al 1992). This has been shown to be independent of blood transfusion and could therefore reflect transmission between patients in dialysis centres perhaps because of inadequate decontamination of equipment.

Organ Transplantation

HCV may be acquired prior to transplantation, from blood transfusion at the time of transplantation (prior to blood donor screening) or from HCV infection in the organ donor. In one study a transmission rate of nearly 50% occurred in patients transplanted from HCV positive donors, (Pereira 1992).

Needlestick Injury

There are a number of case reports describing transmission of HCV infection after occupational needlestick injury or cuts with sharp instruments. (Seef 1991; Marranconi 1992; Cariani 1991). Overall 4-10% of needlestick injuries with HCV infected blood result in transmission of infection. (Alter 1994; Mitsui et al 1992; Kiyosawa et al 1991). Dentists (2%) and particularly those that do oral surgery (9%) are at higher risk of contracting HCV infection. (Klein et al 1991).

The risk factors that seem to be associated with increased rate of needlestick transmission in dental practitioners are type of needle (hollow versus solid), frequency of occupational blood contacts, the type of patient (acute infection versus chronic carriers) and prevalence of HCV among patients (Memon et al 2002).

Other parenteral risk factors.

Other potential parenteral risk factors include tattoos using non-sterile needles, ear piercing and previous immunisation.

Sexual transmission

Many studies of partners of HCV infected patients have been undertaken and rates of infection of 0-15% (average 5%) have been reported. (Bresters et al 1993;Osmond et al1993; Everhartet al 1990; Brettler et al 1992). Among non–IVDU prostitutes prevalence rates of 4-12 % have been found. (Hadziyannis et al 1993, Mast et al 1991). Prevalence rates among homosexual males have been reported between 1-5% and in non-drug using mostly heterosexual populations attending STD clinics prevalence rates of 1-10% have been reported (Alter 1995).

In summary sexual transmission appears to be inefficient and uncommon although if the patient is co-infected with HIV infection the rate of sexual transmission is higher.

Vertical Transmission

Vertical transmission occurs in less than 10% of children of infected mothers. A recent review of published studies of mother to infant transmission found that the factors associated with higher rate of mother-to-infant transmission were maternal co-infection with HIV and history of previous drug abuse. Mode of delivery and prevalence of breast-feeding did not significantly influence rates of mother-to-infant transmission. Viral factors such as HCV-RNA titres and genotype have not been consistently measured in studies and therefore their role is, as yet, unclear.

(Yeung et al 2001).

Sporadic infection

Finally, in up to 20% of patients there is no apparent risk factor for HCV transmission and is referred to as 'sporadic infection'. Some of this group may be those reluctant to admit to previous intravenous drug use. In addition previous immunisation, dental, treatment and alternative medical practices in other countries particularly Africa may account for infection in some of these people. There may also be other routes of transmission of which we are currently unaware.

1.4 Virology

1.4.i HCV genome

The single positive sensed RNA genome of HCV is composed of approximately 9400 nucleotides with a single translational open reading frame (ORF) that spans 90% of the genome (Choo et al 1989, Choo et al 1991, Takamiza et al 1991, Grakoui et al 1993a, Hijikata et al 1993a). A large polyprotein is produced by translation of this ORF; this undergoes proteolytic processing by host cellular peptidases or viral proteinases resulting in at least 9 viral proteins. (Figure 1.1).

The structural proteins C, E1 and E2/NS1 are released from the N terminal region of the polyprotein following cleavage by host signal peptidases (Hijikata et al 1991). The C protein is believed to form the viral nucleocapsid and is highly conserved among HCV isolates (Houghton et al 1991, Okamoto et al 1992). The E1 and E2/NS1 proteins are the putative viral envelope proteins and contain a hypervariable region (HVR), which may be used to identify HCV genotypes and quasispecies. This HVR is

thought to occur by sequential mutation and be important in escape from host immune surveillance resulting in viral persistence (Weiner et al 1992, Simmonds 1995).

The non-structural proteins are cleaved by two virally encoded proteases.

The NS2 protein contains a zinc-dependent metalloprotease, which initiates cleavage at the NS2/NS3 junction (Grakoui et al 1993b, Hijikata et al 1993b). The remainder of the polyprotein is further processed to NS3, NS4a, NS4b, NS5a and NS5b proteins by the virally encoded serine protease contained in the N-terminal portion of NS3 (Hijikata et al 1993b, Tomei et al 1993, Bartenshlager et al 1994,Van Doorn 1994). In addition the NS3 protein has RNA-stimulated NTPase and RNA helicase activity (Van Doorn 1994, Hong et al 1996, Kim et al 1995).

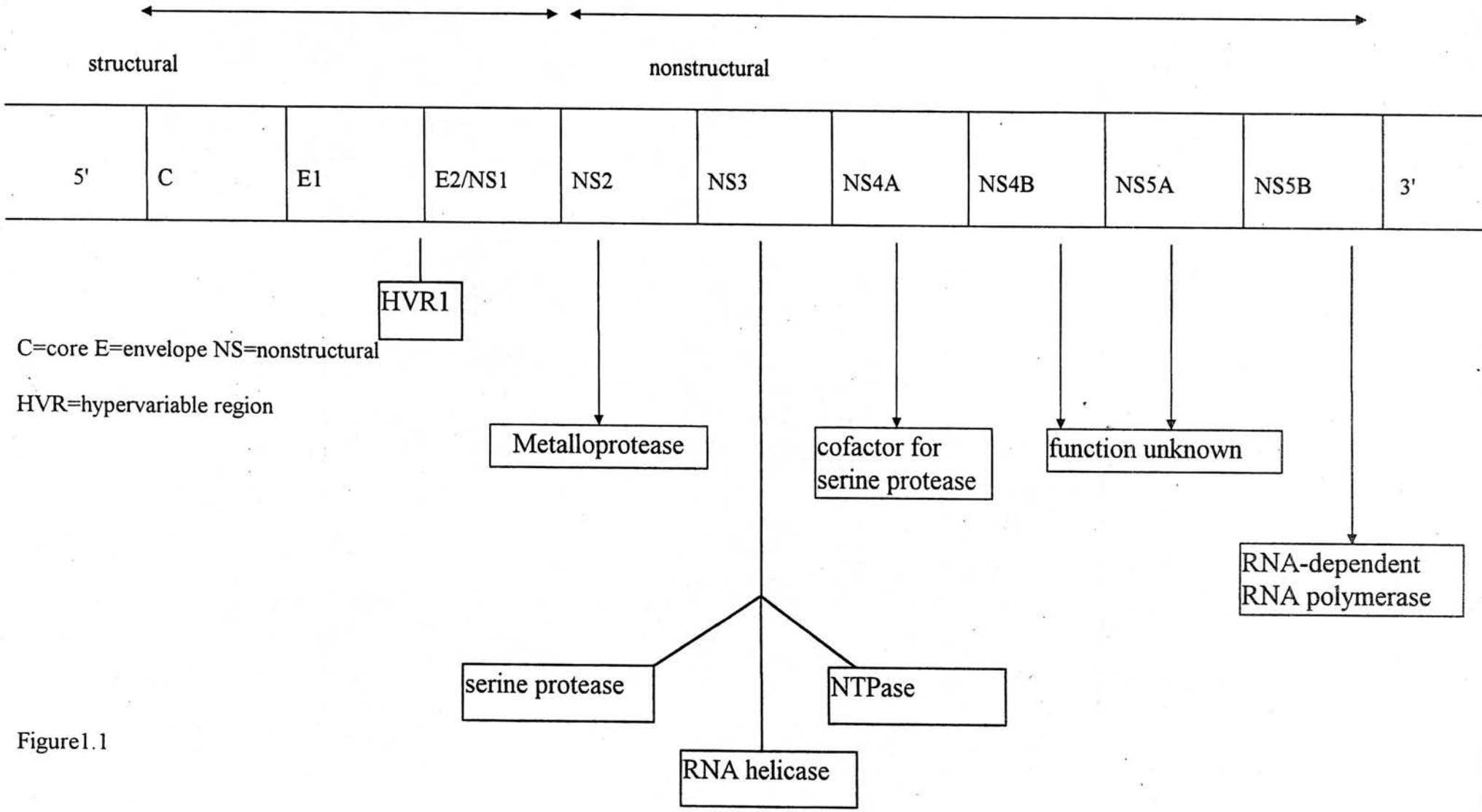

structural
nonstructural
5'
C
E1
E2/NS1
NS2
NS3
NS4A
NS4B
NS5A
NS5B
3'
HVR1
C=core E=envelope NS=nonstructural
HVR=hypervariable region
Metalloprotease
cofactor for serine protease
function unknown
RNA-dependent RNA polymerase
serine protease
NTPase
RNA helicase

Figure1.1

More recently the function of the NS4A protein has been described. Firstly it anchors NS3 to the surface of the endoplasmic reticulum; secondly it is an essential cofactor for cleavage at the NS4B/NS5A and possibly the NS3/NS4A site and finally it enhances cleavage at the NS5A/5B and NS4A/NS4B site (Tanji et al 1995, Lin et al 1995, Shimizu et al 1996).

The mechanism by which this occurs is unknown but the most likely explanation is that the NS4A protein stabilises NS3 in an active formation. The function of the NS4B and NS5A proteins has not been clarified but there is evidence to suggest that the NS5B protein is a RNA-dependent RNA-polymerase essential for replication of the viral genome (Van Doorn 1994, Behrens et al 1996).

At either end of the genome is an untranslated region (UTR). There has been recent interest in the 5' region as the site for an internal ribosomal entry site (IRES). It is likely that translation of the HCV genome is similar to that of picornoviruses where translation is cap-independent and commences at an IRES within the 5' UTR (Tsukiyama-Kohara et al 1992).

1.4.ii genotypes

Sequence comparisons of HCV have allowed the identification and classification of HCV into a series of genotypes that differ from each other in nucleotide sequence. There are at least 6 genotypes identified and each of these may be further divided into more closely related subtypes (Simmonds et al 1995, Bukh 1995).

These exhibit both epidemiological and geographical differences. Genotypes 1,2 and 3 are most commonly found throughout Western countries and the Far East, type 4 is

found predominantly in the Middle East and Central Africa, types 5 in South Africa and type 6 in South East Asia (Simmonds et al 1997).

There has been considerable interest in the clinical significance of these genotypes. With respect to disease progression there have previously been a number of studies suggesting type 1b is associated with a more severe and rapid progression of disease (Booth et al 1995,Watson et al 1996, Kobayashi et al 1996); however other studies have concluded there is no real evidence to support this and it may simply be that type 1b infected individuals have a longer mean duration of infection. (Dusheiko et al 1994, Yamada et al 1994). However there would appear to be more conclusive evidence that type 1b predisposes to the development of hepatocellular carcinoma. (Haydon et al 1997, Tanaka et al 1996). There is also more definitive evidence that genotype affects response to treatment with interferon-alpha and this will be discussed in more detail in section 1.10.i.

1.4.ii quasispecies

In common with other RNA viruses that replicate through an RNA polymerase, HCV circulates as a mixture of closely related genomes termed a quasispecies (Martell et al 1992). This therefore results in the establishment of several closely related but distinct viruses within a single host. Quasispecies are distinct from genotypes in that over the entire genome they show much lower levels of homology. The main clinical importance is related to persistence of infection, and difficulties with developing an effective vaccine. One paper has suggested that more diverse quasispecies are associated with more severe and progressive liver disease. (Honda et al 1994).

1.5 Pathogenesis and host immune response

The exact mechanism of hepatocellular damage in HCV infection is still unknown. Some authorities believe that the virus is directly cytopathic and indeed there is some evidence to support this view.

1.5.i Cytopathic mechanisms

The evidence for this includes:

HCV has some similarities to other members of the flavivirus family (Takamizawa et al 1991, Miller et al 1990) and these viruses including yellow-fever virus are often directly cytopathic.

The observation that alpha-interferon therapy causes rapid normalisation of serum aminotransferases suggests that the drug is acting against a directly cytopathic virus.

Hepatic HCV-RNA levels have also been demonstrated to correlate with the histological activity of HCV infection (Lau et al 1993, Jeffers et al 1993).

However the current opinion is that immune-mediated injury is the main mechanism of hepatocellular injury and that virus-mediated cytotoxicity is less important.

1.5.ii Immune mediated mechanisms.

The host immune response in patients with HCV is important for two reasons. The first is its role in the elimination or attempted elimination of the virus and the second, its role in hepatocellular damage. Both humoral and cell-mediated immune responses have been demonstrated in patients with HCV infection.

Cell mediated immunity

T lymphocytes do not recognise native antigen. The antigen has to be processed, usually by antigen presenting cells, and then presented on the surface of the cell in

association with major histocompatability complexes (MHC) CD4+ T cells recognise antigen bound to Human Leucocyte Antigen (HLA) class II molecules and CD8+ T cells recognise antigen bound to HLA class I molecules.

CD4+ T cells

Most patients have a CD4+ response in the peripheral blood to one or more of the HCV proteins, in particular to core and NS4 (Botarelli et al 1993, Ferrar et al 1994). In the liver CD4+ T cells are present and can respond to both structural and non-structural proteins although the T cell receptors are different to those on the CD4+ cells in the peripheral circulation suggesting compartmentalisation of the T cell response to the site of disease (Minutello et al 1993, Lohr et al 1994).

CD4+ T cells have several functions including production of cytokines, promoting growth and differentiation of cytotoxic T lymphocytes (CTL) and activation of inflammatory cells (Mosmann et al 1986). Patients with HCV infection have elevated levels of these cytokines in serum and liver suggesting a role for these substances in the immune response to HCV (Cacciarelli et al 1995, Reiser et al 1995).

CD 8+T cells

HCV specific CD8+ T cells have been found in both the hepatocytes and peripheral blood mononuclear cells in patients with HCV infection (Kita et al 1993, Koziel et al 1993, Koziel et al 1992, Nelson et al 1996, Tsai et al 1994), but it appears that it is those in the liver which mediate the major CTL response. CD8+ cells may cause direct lysis of virally infected cells or stimulate cytokine-mediated damage. The response seems to be diffuse against epitopes in different structural and non-structural proteins

CTL activity is important in both viral clearance and hepatocellular damage It is therefore interesting to speculate that by attempting to eliminate HCV infected cells, CTLs contribute to hepatocellular damage and that suboptimal or inefficient CTL response results in persistence of infection. Evidence from immunodeficient patients is inconclusive. HCV related chronic liver disease appears to progress more rapidly in liver transplant patients receiving immunosupressant therapy whilst progression in concurrently affected HIV patients is probably not accelerated.

Humoral immunity

The humoral immune response involves antigenic stimulation of B cells with production of antibodies specific to viral proteins. There is evidence that this occurs in HCV infection.

Intraportal lymphoid aggregates have been demonstrated in the livers of people infected with HCV. These consist mainly of activated B lymphocytes in a germinal centre surrounded by a dendritic cell network with a T lymphocyte zone of CD4+ T helper cells and CD8+ T cells (Mosnier et al 1993).

Circulating antibodies are generated against several viral antigens. The development of these antibodies in relation to elimination of HCV is still unclear, but they do not appear to be neutralising. In a similar fashion to cell-mediated immunity the humoral response may contribute to hepatocellular injury.

In conclusion both arms of the immune response are active in patients with HCV infection. However neither appears to be able to eliminate the infection in the majority of patients and it possible that the immune response significantly contributes to hepatocellular damage.

1.6 Histopathological findings in hepatitis C

1.6.i Acute HCV infection

Whilst the pathological findings in chronic HCV are well documented and reviewed there is less information about acute HCV infection. In common with other hepatitis viruses acute HCV induces liver cell injury and necrosis and causes a characteristic response to that injury. On light microscopy ballooning degeneration, focal necrosis and acidophilic bodies are seen. In severe cases necrosis of adjacent liver cells occurs, usually in zone 3 resulting in bridging fibrosis. There is concurrent regeneration of liver cells. Other features that may be seen are mild macrovesicular steatosis and cholestasis. An inflammatory response involving Kupffer cells and lymphocytes is seen. The most severe cases show extensive confluent necrosis producing submassive or massive necrosis.

1.6.ii Chronic HCV infection

The pathological findings in chronic HCV infection whilst characteristic are not specific. The predominant inflammatory cells are the lymphocytes.

The histological abnormalities can be divided as follows: -

Portal tract lesions

Portal tract changes include nodular aggregates of lymphocytes with germinal centres and bile duct lesions

Portal lymphoid aggregates and lymphoid follicles that consist mainly of B-lymphocytes are the characteristic finding in chronic HCV infection. It has been

suggested that these are true functional lymphoid follicles that induce stimulation of effector T cells and B cells. (Mosnier et al 1993).

Bile duct lesions occur in about a quarter of patients with chronic HCV infection. These have similarities to the lesions of primary biliary cirrhosis however the ducts are not destroyed and portal areas without ducts are rarely seen. Small or medium sized bile ducts are infiltrated by lymphocytes and the bile duct epithelium undergoes focal degeneration, necrosis and vacuolation. (Gerber et al 1995).

Piecemeal necrosis

This is thought to be the important pathological process in the progression of chronic hepatitis. It is most easily recognised by irregularity of the limiting plate due to portal inflammation extending through the limiting plate into the periportal parenchyma. (Goodman et al 1995). Unicellular degeneration and necrosis is seen and cell death occurs by apoptosis. As the chronic hepatitis progresses there is continuing erosion of the hepatic parenchyma until small islets of hepatocytes become trapped in expanded portal zones. Fibrosis gradually occurs which progresses to scars. The degree of necrosis is used to grade the degree of activity.

Intralobular lesions

In the hepatic lobules steatosis (micro or macrovesicular), infiltration of sinusoids by lymphocytes, apoptosis and extensive hydropic swelling of hepatocytes are seen. Mild to moderate steatosis is typical of chronic HCV although not universal. (Scheuer et al 1992).

In three reviewed studies bile duct damage was seen in 60%; lymphoid aggregates, follicles or both in 57% and steatosis in 52%. It was surmised that a combination of two out of the above three is seen in at least 50% of patients with chronic HCV and can therefore be useful in the histological diagnosis of this disease. (Gerber 1994, Bach et al 1992, Scheuer et al 1992, Gerber 1995)

Fibrosis and cirrhosis

Chronic hepatitis C appears to be a progressive fibrotic disease although the degree of fibrosis varies from person to person. It is this progressive scarring that leads to cirrhosis and the majority of complications associated with chronic HCV infection.

Progressive piecemeal necrosis results in collagen deposition and sinusoidal cappillarisation. Portal to portal fibrous bridging occurs. In addition central to portal and central-to-central fibrous bands may form. Contraction of theses fibrous bands and nodular regeneration of the surviving parenchyma leads to cirrhosis.

Hepatocellular carcinoma

There is now a significant amount of evidence that chronic HCV infection is a major risk factor for the development of hepatocellular carcinoma. Studies have indicated that 13-80% of patients with HCC have positive anti-HCV antibodies. The mechanism of hepatocarcinogenesis in chronic HCV infection is unknown however it is speculated that the development of liver cirrhosis with continuing hepatocellular necrosis, regeneration and chronic inflammation may be important in the malignant transformation of hepatocytes.

1.7 Natural history of HCV infection

1.7.i Acute HCV

Because acute infection with HCV is usually asymptomatic it is rarely diagnosed. Only a minority of those infected appear to clear the virus and the remainder develop chronic HCV infection with the risks of development of cirrhosis and its complications and hepatocellular carcinoma.

Acute HCV results in malaise, nausea, dark urine and jaundice. Jaundice occurs in less than 20% of those infected and the mean incubation period to onset of symptoms is 7 weeks (range 3-20 weeks). (Alter et al 1989, Barrera et al 1995).

HCV RNA becomes detectable in the serum from1-3 weeks after exposure (Farci et al 1991) and HCV antibody within 6-8 weeks. After 2-5 weeks serum ALT begins to rise and thereafter-clinical signs may become apparent. The severity is variable and only one third of infected patients develop clinical signs and jaundice. It has been observed that those who develop clinically apparent hepatitis seem to clear the virus and therefore avoid the development of chronic disease more frequently than those who do not. (Giuberti et al 1994).

1.7.ii Chronic HCV infection

The natural history of chronic HCV infection is complex. Progression to chronic liver disease occurs in at least 80% of infected patients and the development of cirrhosis in at least 20% of cases by 20 years after infection. (Alter et al 1992, Seeff et al 1992).

Some experts believe that progression of chronic hepatitis C is inevitable from mild chronic hepatitis through moderate to severe histological changes to cirrhosis and then hepatocellular carcinoma however others believe that only a proportion of infected patients will develop progressive disease and the challenge is therefore to predict in whom disease will progress and concentrate treatment on that group.

There have been several studies of natural history to date and some of the most important are highlighted here.

Seeff et al in 1992 studied patients with transfusion associated NANB hepatitis and found no difference in mortality from all causes in those with TAH although there was a small but statistically significant increase in the number of liver related deaths. (Seeff et al 1992)

Alter et al in 1992 studied the natural history of community acquired HCV infection. Their conclusion was that chronic hepatitis developed in a significant proportion of patients with community acquired HCV infection and that HCV infection appeared to persist for at least several years. (Alter et al 1992).

Tong et al in 1995 studied clinical outcomes in patients after transfusion associated hepatitis and concluded that chronic TAH was a progressive disease and in some patients led to death from liver failure or hepatocellular carcinoma. During the follow-up period 20/131 (15.3%) of patients died; 19/20 (95%) from complications of cirrhosis or HCC. (Tong et al 1995).

Di Bisceglie et al prospectively studied patients receiving blood transfusions during cardiac surgery. 53/1070 became positive for anti HCV. They noted progression to cirrhosis in a significant number of cases. (Di Bisceglie 1991)

Niederau et al prospectively studied 838 patients with chronic HCV infection and found an increased mortality compared with the general population mainly when cirrhosis was present and when patients had contracted the disease at less than 50 years of age. (Niederau et al 1998).

More recently a systematic review was undertaken of published epidemiological studies with the aim of estimating the rate of progression to cirrhosis and the factors that affect it. (Freeman et al 2001). The estimated rate of progression to cirrhosis after 20 years of chronic HCV infection was calculated as 22% for patients attending hepatology clinics, 24% for post transfusion cohorts, 4% for blood donor series and 7% for community based cohorts.

Overall it is apparent that the natural history of chronic hepatitis C infection is not as yet fully defined. Current knowledge would suggest that the disease is progressive over a variable time frame and that serious complications do not generally occur until at least 20 years after infection. However it has become clear that there are certain factors that may be important in the severity and rate of progression of liver disease.

Factors affecting progression of HCV-related liver disease

There are multiple factors that have been postulated to be important in HCV induced liver disease. These can be divided into patient factors; virus factors and co-factors (table 1.1).

Patient Factors	Virus Factors	Co-Factors
Increasing age	Increased virus level	HBV infection
Mode of transmission (blood transfusion)	HCV genotype 1b	HIV infection
Duration of infection	Diverse quasispecies	Alcohol abuse
Host HLA status		

Table 1.1: Patient, virus and cofactors affecting severity and progression of hepatitis C infection

Patient factors.

Age at the time of infection appears to be important. Poynard et al in their study on the natural history of liver fibrosis progression found that age at infection was the main risk factor for fibrosis progression. (Poynard et al 1997). There are a number of other studies that concur with this finding that age at time of infection has a significant impact on development of advanced liver disease and liver-related complications. (Tong et al 1995, Kenny-Welsh et al 1999, Freeman et al 2001).

Mode of transmission of infection has also been reported as being relevant. Several authors have found that those infected through transfusion of infected blood had more severe and progressive liver disease. (Alter et al 1992, Gordon et al 1993, Roudot Thoraval et al 1997).

Male sex has been reported as being associated with a poorer outcome. Poynard et al found that male sex was independently associated with fibrosis (Poynard et al 1997). In addition in a recent epidemiological review, male sex was found to be one of the three factors associated with more rapid disease progression (Freeman et al 2001). However several other authors have not replicated this observation other than the association between male sex and the development of hepatocellular carcinoma. (Niederau et al 1998, Fattovich et al 1997).

Host HLA status. There has been considerable interest as to whether certain HLA antigens are important in both the development of chronic disease and the progression of liver disease in chronic HCV carriers. Several antigens have been found to be protective against the development of persistent HCV infection (Congia et al 1996, Zavaglia et al 1996). In addition several alleles have been implicated in the development of cirrhosis. (Aikawa 1996).

Virus factors

Genotypes. Until recently it was believed that genotype 1b was associated with the development of more severe disease. (Dusheiko et al 1995, Booth et al 1995, Kobayashi et al 1996). However more recent studies have found no role for genotype in disease severity and have suggested that the previous findings may be due to the longer duration of disease associated in general with genotype 1b. (Serfaty et al 1998; Niederau et al 1998, Poynard et al 2001).

Virus level. Lau et al found that high HCV-RNA levels were associated with more severe histological changes in the liver. (Lau et al 1993). Similar findings are reported by other groups (Booth et al 1995; Hagiwara et al 1993) but as the level of viraemia fluctuates it is unlikely that a single HCV-RNA level can be entirely predictive of outcome. It has also been speculated that dose of initial innoculum is related to the rate of progression of disease and that this accounts for some of the differences in blood transfusion recipients (Roudot-Thoraval et al 1997). However more research in this area is required.

Diverse Quasispecies. Honda et al examined the degree of diversity of quasispecies in relation to progression of liver disease and found that the degree of diversity of HCV quasispecies was related to the progression of liver disease. (Honda et al 1994). However more recent studies have failed to show any role in progression of liver disease once chronic hepatitis has developed. (Seef et al 2002)

Co factors

HBV appears to exert an additive effect on HCV in the progression of disease. There are a number of reports indicating the role of HBV co-infection in the development of cirrhosis and hepatocellular carcinoma. (Fong et al 1990, Alberti et al 1994, Benvegnu et al 1994).

Co infection with HIV would appear to accelerate the development of liver complications in patients co-infected with HCV (Martin et al 1989, Eyster et al 1993). However HCV co-infection does not influence the rate of progression of clinical end-points in HIV-infected drug users. (Haydon et al 1998).

Alcohol. There has been significant interest in alcohol as a co-factor in chronic HCV disease. It appears likely that the combination of viral-injury and alcohol induced injury predisposes to more severe liver injury than either agent alone. Ostapowicz reported that on multivariate analysis total alcohol consumption was independently associated with the presence of cirrhosis. (Ostapowicz et al 1998). Other authors concur with this with excess alcohol consumption being associated with an increased rate of fibrosis progression (Poynard et al 1997, Roudot-Thoravol et al 1997, Freeman et al 2001) or decreased survival. (Niederau et al 1998). A more recent study reported that a history of heavy alcohol use was strongly associated with an increased risk of developing cirrhosis and that patients with both transfusion associated HCV and a history of heavy alcohol use were 31.1 times more likely to develop cirrhosis than controls with no history of alcohol abuse or HCV infection. Heavy alcohol intake increased the risk of cirrhosis in patients with HCV by approximately fourfold. (Harris et al 2001).

1.7.iii Hepatocellular carcinoma

It is now accepted that chronic HCV infection is a major risk factor in the development of hepatocellular carcinoma. This would appear to be restricted to those with cirrhosis although the development of HCC in patients without cirrhosis has been described. (De Mitri et al 1995, Bralet et al 2000).

Many studies of natural history of HCV infection describe the risk of HCC in HCV infected patients. In many regions HCV infection is the major risk factor identified for HCC with up to 75% of patients testing positive for anti-HCV. (Di Bisceglie 1997). The mechanism by which HCV induces the development of HCC is not known. In HBV infection the DNA becomes integrated into host chromosomes and is thought to have a directly carcinogenic effect. However as HCV is an RNA virus the mechanism is less clear. Alcohol, infection with HBV, male sex and possibly genotype 1b are important co factors in the development of HCC in patients with HCV cirrhosis. Yamauchi reported the 10-year rate of HCC was 19% in those with cirrhosis caused by alcohol alone, 57% in those with HCV-related cirrhosis and 81% among those with HCV infection who consumed more than 120g of alcohol per day. (Yamouchi et al 1993).

The rate of progression to HCC from cirrhosis is more difficult to estimate. Di Bisceglie estimated the risk of development of HCC from results of natural history studies of HCV infection and studies on the development of HCC in patients with HCV–related cirrhosis. He estimated that if 60% of patients exposed to HCV develop chronic hepatitis and 20% of them develop cirrhosis within 10 years, approximately 12% of all patients infected with HCV would have cirrhosis and be at risk of developing HCC. The risk of developing HCC is 1-4% per year which after 20 years

would mean that 1.2%-4% of all infected patients would have developed HCC; 1.9%-6.7% of those with chronic HCV and 9.6%-33.5% of those with cirrhosis would have developed HCC. (Di Bisceglie 1997). Obviously the rate of development of HCC may be higher once additional co-factors such as alcohol were taken into consideration and with increasing duration of infection.

1.8 Extrahepatic manifestations of hepatitis C

Hepatitis C has been found to be associated with a number of extra-hepatic manifestations. These include immunological disorders such as autoimmune hepatitis, Sjogrens disease, glomerulonephritis, essential mixed cryoglobulinaemia and polyarteriris nodosa. (Lunel 1994, Pawlotsky 1994). The exact role of HCV in the aetiology of these conditions is unclear.

There are a number of cutaneous associations of HCV including porphyria cutanea tarda and lichen planus. There have been several different reports indicating the high frequency of anti-HCV antibodies in patients with porphyria cutanea tarda up to 66% in one series. (De Castro et al 1993, Murphy et al 1993).

Other associations include focal lymphocytic sialedenitis, Mooren corneal ulcer and aplastic anaemia.

1.9 Investigation in hepatitis C

Diagnostic Tests for hepatitis C

Diagnostic tests for HCV can be divide into two main groups:

serological tests that detect antibody to HCV

molecular assays that detect HCV RNA in the serum

1.9.i Serological assays

These can be further divided into screening tests and supplemental or confirmatory tests.

Screening tests

Since the cloning of HCV in 1989 serological tests based on antibody detection have been developed for the diagnosis of HCV infection. The main screening test is an ELISA. The first generation antibody test incorporated a single HCV recombinant antigen derived from the NS-4 region designated c100-3. This assay lacked sensitivity and specificity and was succeeded by the second and third generation assays that incorporated other immunogenic areas of the virus. In 1992 the second generation test was introduced which incorporated HCV antigens from the core and NS-3 regions in addition to the NS-4 derived antigen. This resulted in a significantly improved sensitivity but only a slightly improved sensitivity. With the introduction of the third generation ELISA which improved sensitivity further. (Gretch 1997)

ELISA-1	70-80%
ELISA-2	92-95%
ELISA-3	97%

The third generation assay incorporates antigens from the core, NS-3, NS-4 and NS-5 regions. (Figure 1.2.)

However in view of the number of false-positive results particularly in low prevalence groups such as healthy blood donors, introduction of confirmatory tests occurred.

Confirmatory tests

The supplementary assay developed for confirmation of these ELISA tests is the Recombinant Immunoblot assay (RIBA). In this assay HCV antigens are immobilised on nitro-cellulose strips. The RIBA-3 assay is now the most widely used and synthetic peptides from the core and NS-4 regions and recombinant antigens from the NS-3 and NS-5 regions are used. RIBA tests are interpreted as positive if there is reactivity to one or more antigens and indeterminate if reactivity occurs to only one antigen. Indeterminate tests require confirmation with HCV-RNA testing.

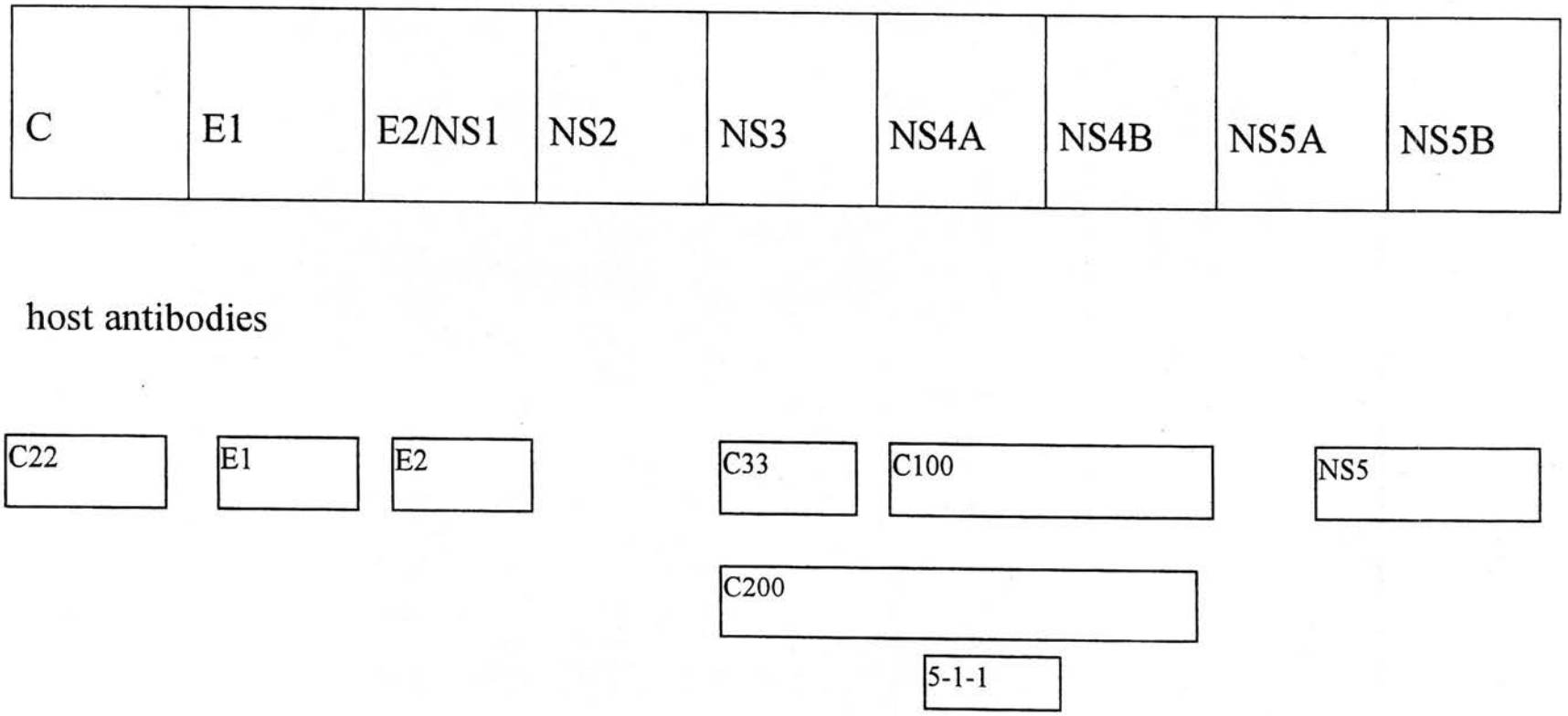


Figure 1.2: Origin of cloned antigens used in anti-HCV diagnostic tests

However in several groups of patients antibody tests may not detect HCV infection. It may take up to 6 months for an anti-HCV response to occur, the mean period for antibody detection is 12 weeks. Also RIBA indeterminate patients need HCV infection confirmed if present. In immunosuppressed patients, HCV infection may occur without detectable antibodies and finally in perinatal transmission antibody tests may not indicate actual transmission of infection. In all these groups detection of HCV viraemia is necessary.

1.9.ii Molecular assays

The use of molecular assays in HCV infection can be divided into three applications

Qualitative tests

Quantitative tests

Genotyping

As HCV –RNA is present in relatively low concentrations in the circulation the nucleic acid has to be amplified. This is done by the polymerase chain reaction (PCR). A portion of extracted viral RNA is transcribed into DNA and the number of DNA molecules is amplified to a detectable level using primers, enzymes and temperature cycling. Using primers from the 5' nontranslated region of the HCV genome increases sensitivity. (Simmonds et al 1990).

The main problem with the technique is standardisation of assays. In one reported survey only 16% of 31 European laboratories scored perfectly in a comprehensive standardised test panel. (Zaaijer et al 1993).

<u>qualitative tests</u>

This basically describes the detection of HCV-RNA by PCR techniques. Optimal HCV-PCR assays have a sensitivity of less than 100 copies of HCV-RNA per ml of plasma or serum.

quantitative tests

This describes the measurement of circulating HCV-RNA and therefore determines the viral load. There are two main methods of doing this. Firstly quantitative PCR where the target is amplified and secondly branched chain DNA signal amplification assays where the signal is amplified rather than the target. The main problem of the PCR technique is that amplification of the HCV-RNA prior to quantification can lead to wide variation of results on the same sample. The main drawback of the branched chain method is the lack of sensitivity. The main uses of the quantitative techniques are in determining viral load, which may be relevant in the natural history of the disease, and for monitoring response to treatment.

Genotyping

The clinical importance of genotypes in HCV infection remains controversial and their use would appear to be most relevant in predicting treatment outcome. (section 1.10.i). Methods of determining genotype are available and are now being more frequently used when assessing patients for treatment.

1.9.iii Liver function tests

Aminotransferases

ALT and AST are still used in the investigation of HCV infection. In acute hepatitis their level is often raised with a similar pattern to other forms of acute hepatitis. However in the chronic setting its value is more controversial. A significant number of patients who are HCV-RNA positive and who have chronic hepatitis on liver biopsy have a normal ALT value (Stanley at al 1996). In addition the level tends to fluctuate and is not directly correlated with the degree of hepatic inflammation. The test is still useful in assessing biochemical response to interferon-alpha therapy: if it normalises during the first 2-3 months of therapy the patients is deemed a responder (if a patient has a normal ALT then RT-PCR for HCV-RNA has to be used to assess response).

1.9.iv Histology

Because of the variable progression and natural history of HCV infection liver biopsy is a useful tool for staging disease and in some cases predicting prognosis. In addition if patients have developed cirrhosis they can be entered into a screening programme for hepatocellular carcinoma. A liver biopsy can aid the decision as to whether interferon-alpha therapy might be of benefit to the patient.

Both the grade of inflammatory activity and the stage of fibrosis are determined. The typical histological findings are discussed in section 1.6.ii.

1.10 Treatment

The definitions of response to treatment are provided in table 1.2

	Non-response	Partial response	Sustained response
ALT	Remains abnormal	Transient decrease or normalisation	Normalisation
HCV RNA in serum by RT-PCR	Remains positive	Initially negative, then reverting to positive	Negative for six months after therapy
HCV level by quantitative PCR	Unchanged	Initial decrease, then reverting to pre-treatment levels	Undetectable

Table 1.2: Definitions of response to treatment in patients with hepatitis C infection

1.10.i Interferon-alpha

Interferons are host proteins produced in response to infection with most viruses. There are three main types of interferon, alpha (produced by B lymphocytes and macrophages), beta (produced by fibroblasts and epithelial cells) and gamma (produced by T lymphocytes that have been sensitised to foreign antigen). Interferons have both antiviral (interrupting viral replication) and immunomodulatory (increasing the number and activity of antibody producing cells and cytotoxic T lymphocytes and also upregulating the expression of cytokine receptors on cells) activity.

Alpha-interferon was the first licensed treatment for chronic HCV infection in the UK and USA. Its precise mechanism of action in this disease is unclear. Its efficacy in the treatment of NANB hepatitis was first analysed in 1986 (Hoofnagle et al 1986). Since then many prospective, multicentre, randomised controlled trials have been carried out. (Table 1.3) (Booth et al 1995, Hagiwara et al 1993, Saracco et al1993, Reichard et al 1994, Jouet et al 1994, Poynard et al 1995, Chemello et al 1995, Yamada et al 1995, Kasahara et al 1995,Sanchez-Tapias et al 1996).

Summarising these data, the response rate to a standard regimen (3 megaunits thrice weekly for six months) of interferon therapy is 50%. However at the end of treatment 50% of these responders will relapse giving an overall sustained response rate of 25%. In the earliest trials, response to interferon alpha was assessed only by normalisation of serum aminotransferases. More recently virological cure has been defined as an absence of HCV RNA from liver tissue, serum and peripheral blood mononuclear cells although a disparity between biochemical and virological response is not uncommon (Lau et al 1993, Pawlotsky et al 1994).

Centre	Dose (MU/TIW)	Duration of therapy	Number of patients	Sustained response (SR) (Biochemical)	Sustained response (Virological)	Associations with a poor response	Comments
Osaka, Japan	3	6	26	15.4%	7.7%	high pre-therapy HCV-RNA titre	One of the first studies to include HCV-RNA titre as a measure of response
Hagiwara et al 1993	6-3	6	27	29.6%	25.9%		
Torino, Italy	3	6	26	15.4%	}	cirrhosis	Unfortunately the virological SR rate for each group of patients was not given.
Saracco et al	1	6	29	13.8%	}		
1993	3	12	14	57.1%	} 12.5% overall		
	1	12	11	9.1%	}		
Huddinge, Sweden	3	12	40	37.5%	42.5%	long disease duration	This demonstrated a higher SR with longer duration of therapy
Reichard et al 1994							
Creteil, France	3	6	52	13.5%	n/a	cirrhosis	The first study to show that cirrhotic patients have a decreased SR rate
Jouet et al	3-1	12	58	28.6%			
1994							
France	3	18	103	22.3%	n/a	cirrhosis increased age	Large multicentre study illustrating the benefit of increased duration of treatment
Poynard et al	3-1	18	101	9.9%			
1995	3-0	6	99	8.1%			
Italy	6	12	59	49%	42.4%	genotype 1b longer disease duration	Multicentre trial. Concluded that SR rate is affected by dose and duration of therapy particularly in patients with genotype 1b
Chemello et al	3	12	61	31%	24.6%		
1995	6	6	54	28%	25.9%		

Okayama, Japan **Yamada et al** **1995**	5-2.5	6	60	38.3%	n/a	genotype 1b HCV-RNA titre severe disease on biopsy	
Osaka, Japan **Kasahara et al** **1995**	5-2	6 12	45 43	33.3% 53.5%	n/a	genotype 1b advanced liver fibrosis	This study reiterates that infection with genotype 1b is associated with a poor response to therapy
London, UK **Booth et al** **1995**	5-0.5	12	20			persistence of detectable serum HCV-RNA during treatment	This study concluded that persistence of HCV viraemia at three months was associated with failure of therapy.
Barcelona, Spain **Sanchez-Tapias et al** **1996**	5-1.5	12	141	15.6%		not exposed through ivdu older age more severe disease serotype 1	This trial compared different treatment regimes. The dose was progressively reduced if response occurred and progressively increased if non-response occurred. It concluded that some patients were very sensitive to interferon therapy and would only require a small dose of the drug to attain a SR but that other patients were refractory to treatment despite increasing doses.

Table 1.3: Summary of trials of interferon-alpha therapy

The initial recommendation for alpha-interferon therapy was three megaunits subcutaneously, three times weekly for six months. There was, however, good evidence that increasing the duration of treatment resulted in higher sustained response rates (Saracco et al 1993, Poynard et al 1995, Kasahara et al 1995, Di Bisceglie et al 1992, Kobayashi et al 1992). However increasing the dose above 3 megaunits does not appear to confer any therapeutic benefit. (Saracco et al 1990, Davis et al 1989, Marcellin et al 1991, Saez-Royvela 1991).

There are other important factors affecting response to treatment. Infection by HCV genotype 1 (Kasahara et al 1995, Yoshioka et al 1992, Mita et al 1994), a high virus level prior to therapy (Hagiwara et al 1993, Magrin et al 1994, Shibata et al 1993) and the presence histologically of cirrhosis (Jouet et al 1994, Camps et al 1993) all appear to significantly reduce the rate of sustained response. Monotherapy has now been almost completely superseded by combination therapy although there are a group of patients in whom ribavirin is contraindicated and who therefore continue to be treated with monotherapy.

There has been considerable debate as to whether interferon alpha can modify the natural history of HCV related cirrhosis. There have been conflicting results as to whether interferon can prevent or delay the development of hepatocellular carcinoma even inpatients who fail to respond to treatment (Nishigucchi et al 1995, Mazella et al 1996). Further studies have examined the effect of interferon therapy on preventing other complications of cirrhosis. (Benvegnu et al 1998, Fattovich et al 1997, Camma et al 1998). Many of these studies were retrospective. However a more recent prospective trial was carried out with the aim of assessing the impact of interferon treatment on the natural history of HCV cirrhosis. (Gramenzi et al 2001). This trial

found that the natural history of cirrhosis was not significantly affected by interferon treatment neither was it associated with an improvement in survival. However there was a significant reduction in the cumulative incidence of hepatocellular carcinoma in the treated patients when compared to the untreated group. The impact of treatment was even greater in those patients achieving a sustained response.

There are several side effects known to occur with interferon-alpha therapy (table 1.4) The mechanism of these is unclear.

Side-effects appear to have a dose response relationship. Identification of patients at risk of severe side effects is important. In particular patients with underlying psychiatric disorders have a high risk of neuropsychiatric complications of treatment, which can be severe and associated with psychotic and suicidal thoughts.

Pegylated interferon

During the last two years long-acting interferons have been developed. The addition of a polyethylene glycol (PEG) molecule to interferon alpha has resulted in a product with better physical and thermal stability. This results in a longer half-life and enables the interferon to be given once weekly. There are 2 PEG-interferons currently in use. The first has a 12kDa linear PEG tail attached to interferon alpha2b and the second has a 40kDa branched PEG molecule attached to interferon alpha2a. Used as monotherapy both types are associated with an increased SR compared to standard interferon alpha. (Lindsay et al 2001, Zeuzem et al 2000). In addition the PEG interferons have a similar occurrence and range of side-effects as standard interferon-alpha.

SIDE EFFECTS OF INTERFERON ALPHA

EARLY

Flu-like illness, chills, fever, malaise, muscle aches, headache

Poor appetite

LATER-COMMON

Weight loss

Increased need for sleep

Psychological side effects (irritability, anxiety, depression)

Hair loss

Thrombocytopenia, leucopenia

UNUSUAL OR SEVERE

seizures

acute psychosis

bacterial infections

autoimmune reactions

hyperthyroidism or hypothyroidism or transient thyroiditis

RARE

proteinuria

cardiomyopathy

rashes

interstitial lung disease

retinal changes

ototoxicity

Table 1.4: Side-effects of interferon-alpha therapy

1.10.ii Alternative treatments

Because of the rate of relapse after interferon therapy the use of other agents both alone or in combination has been examined.

Ursodeoxycholic acid

Ursodeoxycholic acid (urso) is a hydrophilic and non-toxic bile acid that has been reported to reduce ALT in a number of chronic liver diseases e.g. primary biliary cirrhosis. (Leuschner et al 1989, O'Brien et al 1991, Plevris et al 1991).

In HCV infection treatment with urso has been shown to result in a significant reduction in serum ALT, however this was followed by a return to pre-treatment ALT levels during follow-up. HCV-RNA titres were unaffected. (Puoti et al 1995, Attili et al 1994). Combination treatment with urso and alpha-interferon does not show an improvement in SR when compared with interferon treatment alone. (Angelico et al 1995, Boucher et al 1995).

Iron depletion

There are many reports of chronic HCV infection being associated with an elevated hepatic iron content (Di Bisceglie et al 1992), and it has been postulated that the efficiency of HCV therapy may be influenced by the amount of hepatic iron present (Van Thiel et al 1994, Girelli et al 1995, Olynyk et al 1995). In addition it has been reported that serum ALT levels decrease after phlebotomy alone in HCV infection. However HCV-RNA levels were not assessed in this study and there was no evidence of any histological improvement (Hayashi et al 1994).

NSAID

Alpha-interferon plus ketoprofen (a NSAID) as combination therapy has been demonstrated in a single study to produce an improved sustained response rate compared with alpha-interferon alone (Andreone et al 1995), although no further studies have reported similar findings.

Amantadine

Amantadine is an antiviral agent that has been used to treat influenza virus and has also been shown to have activity against flaviviridae. Previous studies have suggested that amantadine may have some effect against HCV either alone or in combination with interferon. A recent study however found no significant increase in SR when treatment with amantadine and interferon was compared with interferon monotherapy (Caronia et al 2001).

Thymosins

Thymosins are hormone-like polypeptides processed by thymus epithelial cells. They regulate maturation of T lymphocytes, stimulate CTL as well as CD3+ and CD4+ lymphocytes, modulate interferon production and stimulate the expression of Interleukin-2 and its receptor.

Bovine thymus extract, which has similar properties to thymosin, has been used alone in the treatment of HCV. Although it causes a reduction in aminotransferases there was no significant sustained response rate (Civeira et al 1989). In combination with alpha-interferon it seems to improve the overall sustained response rate (Sherman et al 1994). Zadaxin is an immunostimulant originally developed for the treatment of

hepatitis B infection. It is composed of thymosin alpha-1 and alpha-interferon. A phase III clinical trial conducted in Italy reported that 47% of patients treated with combination therapy achieved a sustained response (both virologically and biochemically) (Sciclone pharmaceuticals press release). Thymosin has been well tolerated with no evidence of toxicity or drug-related side effects in over 1000 patients studied. A second phase III trial in the US produced disappointing results and the development of the product was discontinued

Ribavarin

Ribavirin is a guanosine analogue with a broad spectrum of action against RNA and DNA viruses including the flavivaridae (Patterson et al 1990). It is thought to inhibit the normal transcription of mRNA although its mechanism of action in HCV is unclear. Several studies have demonstrated that although serum ALT is significantly reduced during treatment with ribavirin, six months after withdrawal of therapy the serum ALT returned to its previous level. In addition HCV-RNA levels do not change during or after treatment. Finally, there is no significant histological improvement after ribavirin therapy. (Di Bisceglie et al 1992, Reichard et al 1991, Camps et al 1993, Dusheiko et al 1994, Bodenheimer et al 1994).

Combination therapy with ribavirin and alpha-interferon however has offered new prospects in the treatment of chronic HCV infection and will be discussed in the next section.

The modes of action of the drugs used in the treatment of chronic HCV infection are summarised in table 1.5.

	Host	Virus	Notes
Alpha-interferon	-↑number of Ab producing cells -↑number/activity of T-lymphocytes -Upregulation of cytokine receptor expression	Effect on viral replication minimal in chronic HCV infection	Exact mechanism of action unknown in HCV infection
Ribavirin		Inhibits transcription of viral RNA	Exact mechanism of action unknown in HCV infection
Ursodeoxycholic acid			Mechanism unclear; may prevent damage to hepatocytes by endogenous hydrophilic bile acids or may have direct cytoprotective effects on the hepatocyte
Protease inhibitors		-Inhibition of HCV serine protease and mettaloprotease required for polyprotein processing	
Thymosins	-Regulation of T-lymphocyte maturation -Regulation of lymphocyte production -Stimulation of Il-2 expression		

Table 1.5: Mechanisms of drugs used in chronic HCV infection

1.10.iii Combination therapy with ribavirin and interferon

In 1998 4 large international multicentre studies reported that combination therapy with alpha-interferon and ribavirin was significantly more effective than interferon monotherapy. Reichard et al found that 36% of interferon naïve patients treated with combination therapy for 34 weeks had a sustained response rate compared with 18% in the monotherapy group. ($p<0.05$). (Reichard et al 1998). Poynard et al reported a sustained response rate of 43% in their interferon-naïve combination therapy group (treated for 48 weeks) versus 19% in the monotherapy group ($p<0.001$). They also found no significant difference in response rates between patients treated for 48 weeks when compared to those treated for 24 weeks. (Poynard et al 1998).

However logistic regression identified 5 factors significantly associated with response and in patients with fewer than 3 of these factors the odds ratio of sustained response was 2.6 (95% CI 1.4-4.8; $p=0.002$) for the 48 week regime compared with 24 weeks of the combination treatment. implying that patients with fewer favourable factors benefit more from extending the duration of therapy to 48 weeks. McHutchison et al reported a sustained response rate of 31% in patients treated with combination therapy for 24 weeks versus 38% in those treated with combination therapy for 48 weeks versus 6% who received interferon monotherapy for 24 weeks and 13% in those with interferon monotherapy for 48 weeks. ($p<0.001$ for the comparison of interferon alone with both 24 weeks and 48 weeks of combination therapy). (McHutchison et al 1998). Finally Davis et al treated patients who had previously relapsed after interferon monotherapy. The sustained response in the combination group was 49% versus 5% in the monotherapy group ($p<0.001$). (Davis et al 1998).

Lower viral load was associated with higher rate of response in both treatment groups.

Results of studies of combination therapy with PEG interferon are now being reported. The initial two studies report broadly similar findings. Manns et al report a SR of 54% for 48 weeks Rx with PEG interferon plus ribavirin versus 48% for standard combination therapy. (Manns et al 2001). Fried et al report 56% versus 45% for PEG combination therapy versus standard combination therapy. (Fried et al 2001). Of note the overall incidence of side effects was comparable for both groups of treatment.

The genotype of the patient is important when deciding on duration of treatment. It is now clear that those with genotype 1 respond less favourably to treatment than those with genotype 2 or 3. It has been shown that for patients with genotype 2 or 3, 24 weeks of treatment appears to be as effective as 48 weeks. For those with genotype 1 the best chance of SR comes with completing 48 weeks of treatment. The data for combination therapy for genotype 1 with PEG interferon and ribavirin is not yet available but is likely to represent a significant improvement on SR rates in this more difficult to treat group of patients.

In summary with all the currently available data, patients with genotype 1 will benefit from 48 weeks of combination therapy probably with PEG interferon whereas those with genotype 2 or 3 probably only require 24 weeks of therapy.

1.11 Liver transplantation

End-stage cirrhosis secondary to chronic HCV infection is currently an indication for orthotopic liver transplantation (OLT). The number of transplants undertaken for

complications of chronic HCV infection is increasing world-wide. Unfortunately there is a high (perhaps-universal) frequency of recurrence after OLT presumably because of extrahepatic sites of HCV replication, which result in reinfection of the allograft (Konig et al 1992;Feray et al 1994;Weinstein et al 1995). Early studies suggested that despite a high rate of recurrent hepatitis, rates of graft survival were comparable with those in other patients who had undergone OLT. However, a subsequent study (Gane et al 1997) suggested that after extended follow-up, HCV infected patients may develop more problems than patients who have undergone OLT for other indications. There is agreement that HCV genotype 1b is associated with an accelerated and more aggressive graft hepatitis, (Feray et al 1995) but the significance of this in terms of overall prognosis is unclear.

Although OLT increases the range of therapeutic options in chronic HCV infection, reinfection of the transplanted allograft is probably universal and continued monitoring of the disease is mandatory. Strategies of antiviral therapy after liver transplantation are proving problematical as alpha-interferon may increase the rate of graft rejection.

1.12 Vaccine Development

Infection with HCV leads to viral persistence in over 80% of patients despite a cellular and humoral immune response to the virus. Quasispecies develop, which are an attempt by the virus to evade the host's immune system.

In many viral infections the development of neutralising antibodies is associated with recovery from infection and the induction of neutralising antibodies by vaccination is sufficient to protect against viral infection. This does not seem to be the case in

chronic HCV infection. Antibodies generated against the nucleocapsid region, NS3 and NS4 of the HCV genome are present in most people with chronic HCV infection therefore they are unlikely to be neutralising.

Chimpanzees convalescent from infection with HCV are not protected against challenge with homologous or heterologous HCV strains. This again suggests the absence of a neutralising antibody response (Farci et al 1992).

Passive immunisation using HCV immunoglobulin in infected chimpanzees has been demonstrated to prolong the incubation period of acute HCV infection but does not prevent or ameliorate the HCV infection (Zuckerman 1995). Active immunisation using recombinant DNA technology has been investigated. One company have used a vector vaccine to attempt to induce neutralising antibodies against the putative envelope proteins of HCV. Recombinant vaccinia viruses expressing the E1 and E2 proteins of HCV were used to infect mammalian HeLa cells in culture to produce recombinant viral proteins. Five out of seven chimpanzees immunised with this vaccine 30 to 40 weeks prior to challenge with a homologous HCV strain remained disease free whilst in a control group all four chimpanzees developed hepatitis (Choo et al 1994). There are however several limitations to this study. The challenge with the virus was performed at the peak level of antibody production; the challenge dose was a relatively low innoculum of virus and most importantly the question of protection against heterologous strains was not addressed. Vaccine development is underway however an effective vaccine will take more time because of the quasispecies nature of HCV and the unanswered questions that exist about the role of the host immune response to HCV.

1.13 HEPATITIS G

It became apparent after the development of serological assays for hepatitis C virus infection that about 20% of cases of community-acquired hepatitis (Alter et al 1989) and 10% of transfusion associated hepatitis were not related to HCV infection (Alter et al 1991). The search for further agents therefore continued. Two novel blood-borne viruses were discovered independently by two groups of investigators and named hepatitis GB virus C (Simons et al 1995, Leary et al 1996) and hepatitis G virus (Linnen et al 1996). They have significant homology at the nucleotide and amino acid level and are therefore thought to be isolates of the same virus. In this section the viruses will be referred to as HGV.

1.13.i Prevalence

HGV was detected in 1.7% of blood donors in the USA (Linnen et al 1996) and 1.34% in German blood donors (Roth et al 1997). In our own Scottish blood donor population the prevalence was higher with a reported rate of 2.25% in regular blood donors. This study is presented later in this thesis.

In haemodialysis patients prevalence rates of 3.1-57.5% have been reported. (Masuko et al 1996, De Lamballerie et al 1996).

With respect to fulminant hepatic failure HGV has been found in 0-50 % of patients however it is possible that HGV could have been transmitted by transfusion in this ill group of patients (Kuroki et al 1996, Sallie et al 1996)

In intravenous drug users in Japan co-infected with HCV 24% also had HGV detected. (Aikawa et al 1996a).

In homosexual males 31% were HGV-RNA positive and in prostitutes a prevalence rate of 18% was reported. Both of these groups had denied previously injecting drugs. (Scallan et al 1998).

In recipients of plasma products 14%of those who received non-virus inactivated products were positive for HGV-RNA (Jarvis et al 1996). In another study of haemophiliacs 25% of patients were HGV-RNA positive compared with 58/68 being HCV positive. (Hanley et al 1998).

However in a study of the prevalence of HGV in patients with community-acquired non A-E hepatitis only 9% of patients were positive for HGV-RNA. In addition it was felt that the hepatitis in these cases might not be attributable to HGV. (Alter et al 1997).

The prevalence of HGV in chronic liver disease has also been investigated. Approximately 5% of cases of chronic liver disease have a non-A-E cause. Approximately 10% of these patients are positive for HGV-RNA. However HGV RNA is detected as often or more often in patients who are infected with HBV, HCV or both and when it accompanies chronic hepatitis C infection HGV does not affect the severity of liver disease. (Miyakawa et al 1997).

1.13.ii Virus identification

HGV was isolated from a patient with non-A, non-B chronic hepatitis who was later found to be infected with HCV. (Linnen et al 1996). GBV-C was identified using primers from GBV-A and GBV-B, two viruses cloned from the serum of tamarinds

innoculated with serum from a surgeon (GB) with acute sporadic hepatitis (Simons et al 1995). They are both positive single-stranded RNA viruses with a genomic organisation similar to that of the flaviviridae and they are distantly related to hepatitis C (they have 25% homology at nucleotide level). They share 86% of their nucleotides and 96% of deduced amino acid sequences and are considered to be isolates of the same virus.

Detection of HGV in serum relies on PCR amplification of HGV-RNA by reverse transcriptase PCR. In the absence of accurate and reliable serological tests it is unclear whether HGV persists in all infected patients although there are several reports of HGV persisting for many years even up to 17 years. (Hanley et al 1997, Karayiannis et al 1997, Masuko et al 1996).

1.13.iii Transmission

There certainly appears to be parenteral transmission of this virus as the prevalence rates above indicate. Transmission of HGV has been identified through blood and blood product transfusion, intravenous drug use and haemodialysis.

Vertical transmission has also been reported (Feucht et al 1996).

Sexual transmission is more controversial although in the study of homosexual males and prostitutes reported previously the prevalence was significantly higher than that in asymptomatic blood donors (Scallan et al 1998).

1.13.iv Natural history

Prospective studies of transfusion-associated infections have shown that HGV RNA can appear in blood recipients who were negative for the agent before transfusion; transmission from donor to recipient has been clearly established. (Alter 1996). However what is more difficult to elucidate is whether HGV causes hepatocellular injury and indeed whether it is truly a hepatitis virus. Alter et al in a prospective study showed that about 75% of HGV-infected transfusion recipients had no biochemical evidence of liver disease. They also reported that in the few cases of hepatitis in which HGV was the only agent identified the ALT elevations were quite small and there was not a clear relationship between ALT levels and HGV-RNA levels. He further reports that in patients co-infected with HCV and HGV, HGV has no influence on clinical outcome and the ALT levels parallel those of HCV-RNA rather than HGV-RNA. (Alter 1996). In a further study the relationship between HGV infection and ALT levels in patients who were HGV-RNA positive and who had no other aetiological factors for acute or chronic liver disease was studied. 45% had normal ALT levels and 55% had elevated levels. Of those with raised ALT, around a third had ALT levels just above the upper level of normal. It was assumed that the chronic hepatitis in this group was attributable to HGV infection. (Karayiannis et al 1997). However several other groups have now reported absence of liver disease in patients with HGV infection. (Neilson et al 1996, Masuko et al 1996, De Lamballerie X et al 1996).

Alter in his paper makes several observations. Firstly that most patients with prospectively observed HGV infection have no evidence of resulting liver disease.

Secondly that although a number of cases of acute HGV infection were reported in the community acquired study of HGV infection, (Alter et al 1997) the persistence of

viraemia after clinical and biochemical recovery may indicate that the patients were chronic carriers of HGV who had an acute unrelated episode of hepatitis.

Finally in studies of non-A-E hepatitis the prevalence of HGV is in the range of 3-15% higher than in the blood donor population but similar to the rate in patients with nonviral chronic liver disease (Alter 1996).

We can therefore currently conclude that there is no direct prospective evidence to indicate that HGV causes acute or chronic hepatitis. However there is still the possibility that HGV is a virus that may cause hepatitis under certain circumstances.

CHAPTER 2

THE EDINBURGH HCV COHORT: EPIDEMIOLOGY AND PROGRESSION OF DISEASE

2.1 **Introduction**

Chronic HCV infection has a worldwide prevalence of at least 1% (Alter 1995). The natural history of chronic HCV infection is complex. Progression to chronic liver disease occurs in at least 80% of infected patients and the development of cirrhosis in at least 20% of cases by 20 years after infection. (Alter et al 1992; Seeff et al 1992). There is much interest as to what factors may be important in the development of chronic HCV infection and in progression of the disease.

The aim of this chapter is firstly to examine and define the South East of Scotland population of HCV infected patients referred to our unit in Edinburgh and secondly examine progression to cirrhosis in our cohort of patients and identify which factors are important.

2.2 Subjects and methods

2.2.i database

This epidemiological study involved the collection and analysis of data relating to patients presenting to our unit with a diagnosis of HCV infection between October 1995 and October 1997. All patients attending our clinic during this time who were HCV ab positive were entered into the database. Patients were referred from a variety of sources including local GPs, the community drug problem service, the infectious diseases unit and the GU medicine clinic.

A standard proforma was used. (appendix 1).

Demographic details

Demographic details (including age, sex, place of referral) were recorded at the time of the initial clinic appointment and at the time of biopsy.

Route of and duration of infection

Route of infection was classified into the following groups: IVDU, blood or blood product transfusion, needlestick transmission, sexual transmission and sporadic.

Duration of infection was calculated as the time from age at infection to time of biopsy.

Alcohol

Consumption of alcohol was also recorded. Patients were asked directly at clinic what their consumption of alcohol had been per week for the previous five years. They were then classified into one of three categories:

<21 units/week= minimal alcohol intake

21-50 units/week= moderate alcohol intake

>50 units/week= heavy alcohol intake.

Laboratory investigations

Parameters measured included:

Standard LFTs including bilirubin, ALT, alkaline phosphatase and gamma gluteryl transferase.

HCV Ab status (by second generation enzyme immunoassayEIA-2, Abbot Laboratories, Weisbaden, Germany) and third generation recombinant immunoblot assay (RIBA-3, Chiron, Emeryville, California)

HCV-RNA status. This was performed by the University of Edinburgh virology lab. RNA was extracted from 0.1ml of serum from each patient as previously described (Jarvis et al 1994). Briefly, the RNA was pelleted by centrifugation at 100,000g for 90 minutes at 4°C and incubated at 37°C for 2h with 1mg/ml proteinase K in the presence of 40μg/ml of polyadenylic acid, 0.5% dodecyl sulfate, 0.1M NaCl, 50nM Tris-HCl (pH8.0) and 1mM of EDTA. RNA was extracted with phenol; after centrifugation, the supernatant was extracted successively with phenol:chloroform (1:1) and chloroform:isoamyl alcohol (50:1). Nucleic acid was precipitated by the addition of one-tenth volume of sodium acetate (pH 5.2) and 2vol ethanol. The dried pellet was resuspended in 25μl of diethyl pyrocarbonate treated water. RNA was then reverse transcribed and amplified using nested primers matching the 5′-NCR (Chan et al 1992). Products were cleaved with restriction enzymes and the fragments separated by agarose gel electrophoresis using 4% Metaphor agarose.

HCV genotyping. This was performed by Dr Geoff Haydon. RNA was reverse transcribed and amplified using nested primers matching the 5'-NCR. Product DNAs were cleaved with restriction enzymes. The fragments were separated by agarose gel

electrophoresis. Phylogenetic comparisons of sequences in the conserved region of the genome confirm that 5'-NCR can be used to distinguish the six major genotypes. (Haydon et al 1998, McOmish et al 1994)

Serological markers of HBV infection were detected with standard assays (RIA, Abbott laboratories, Weisbaden, Germany).

HIV testing was performed using a third generation plus enzyme immunoassay (Abbott, IL) with confirmation by Biokit ELISA (Biokit, Spain).

Histology

Liver biopsy specimens were assessed by two specialised liver pathologists.

Activity was graded according to intensity of necroinflammatory lesions (nil, mild moderate, severe) (Bedossa et al 1996).

The stage of fibrosis was assessed on a 5 point scale. 0=no fibrosis, 1=portal spurring, 2=pericellular fibrosis, 3=bridging fibrosis and 4=cirrhosis. This scoring system is based on the METAVIR scoring system which has previously been shown to be highly reproducible among pathologists. (Bedossa et al 1994).

Treatment

Details of patients' treatment was recorded including dose, duration, response, side effects and reasons for terminating treatment

Follow up

Follow up details were recorded prospectively including development of complications relating to liver disease, OLT and death.

2.2.ii analysis

Patients included for analysis were those that were both HCV-Ab and HCV-RNA positive. Those that were HCV-RNA negative or that had data pertaining to HCV-RNA status missing were excluded from analysis.

2.2.iii statistics

The chi-squared test and students t test were used to compare frequencies and means. The cumulative probability of developing cirrhosis was calculated using the method of Kaplan and Meier. All patients with biopsy data available and a known duration of infection were included. Analysis of cirrhosis development was performed by computing survival curves according to the Kaplan-Meier method. Development of cirrhosis was considered as the end point. Curves were compared statistically using the log rank test. Multivariate analysis of factors for development of cirrhosis was calculated by the stepwise forward Cox regression model.

2.3 Results

2.3.i Epidemiological results

HCV RNA STATUS

A total of 262 patients were initially included in the database. 214 were HCV-RNA positive, 35 were HCV-RNA negative and in 13 patients the HCV-RNA status was unknown. Only those patients that were HCV-RNA positive were included in the epidemiological analysis i.e. the study population comprises the 214 patients who were HCV-RNA positive.

AGE

There was data regarding age at time of infection for 161/214 patients. The date of infection for injecting drug users was taken as the date of first injection. It has been previously demonstrated that the majority of IVDU contracted HCV in their first year of injecting drug use. The date of exposure to a known risk factor such as blood transfusion was taken as date of infection in the remainder of the patients. Patients who contracted HCV infection through heterosexual, or sporadic routes make up the majority of patients with unknown date of infection. The mean age at time of infection in our patients was 25.2 (+/- 12.2) years.

SEX

In our cohort of 214 patients the sex difference was male 139; female 75.

MODE OF TRANSMISSION

The most common mode of transmission of infection in the cohort of patients is intravenous drug use. The histogram demonstrating the modes of transmission is shown in figure 2.1. The miscellaneous group includes needlestick injury, tattoos, ear piercing and dental treatment.

There is a significant difference in the sex of patients who have contracted HCV through drug use (with a significantly greater number of males than females ($p<0.05$)). There is no significant difference in sex between patients who contracted HCV infection through blood transfusion.

mode of transmission of infection

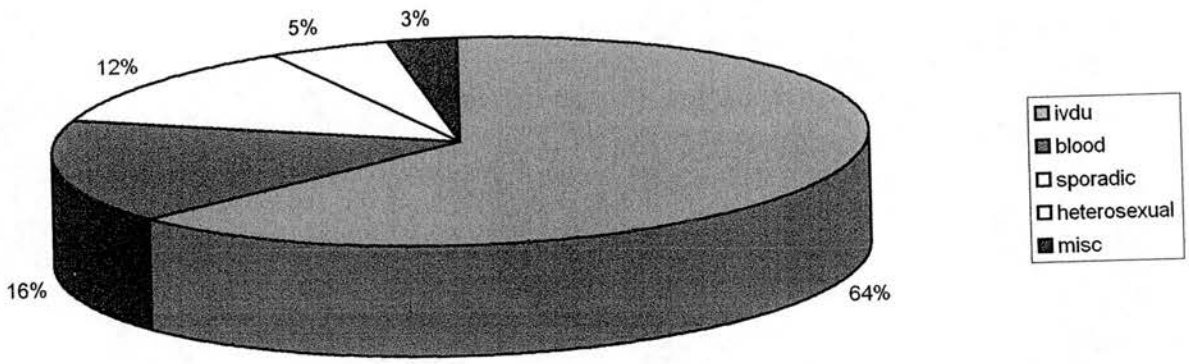


Figure 2.1

Mode of transmission by sex

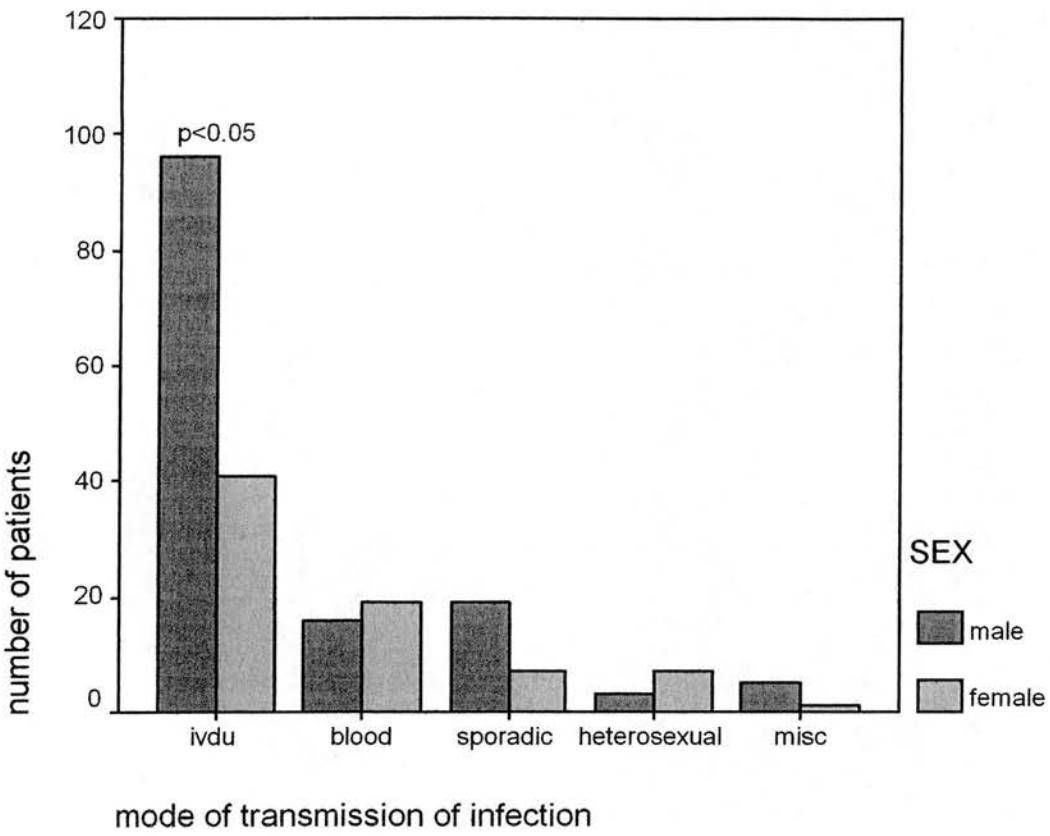


Figure 2.2

ALCOHOL

Alcohol consumption has particular relevance with regards to progression of disease and development of cirrhosis. The alcohol consumption was classified as the amount that had been consumed per week for at least five years. Three categories were defined: minimal intake <21 units per week, moderate intake 21-50 units per week and heavy intake greater than 50 units per week.

50.3 % of our patients were drinking less than 21 units of alcohol per week, 14.4% were drinking between 21 and 50 units per week and 35.3 % were drinking greater than 50 units per week.

There was a significant difference between alcohol consumption and sex. Males were found to drink significantly more alcohol than females ($p<0.001$). (Figure 2.3).

COINFECTION WITH OTHER VIRUSES

16.7% of the HCV infected patients were coinfected with HIV. With regards to hepatitis B infection, 69.9% had no markers for hepatitis B infection; 2.3% and <1% were s antigen and e antigen positive respectively. 27.7% had evidence of previous infection.

ALT VALUES

At initial clinic attendance 15.5% of patients had a normal ALT value (defined as less than 40iu/l). 37.2% had levels up to twice normal and the remainder (47.3%) had values greater than twice normal.

Alcohol consumption by sex

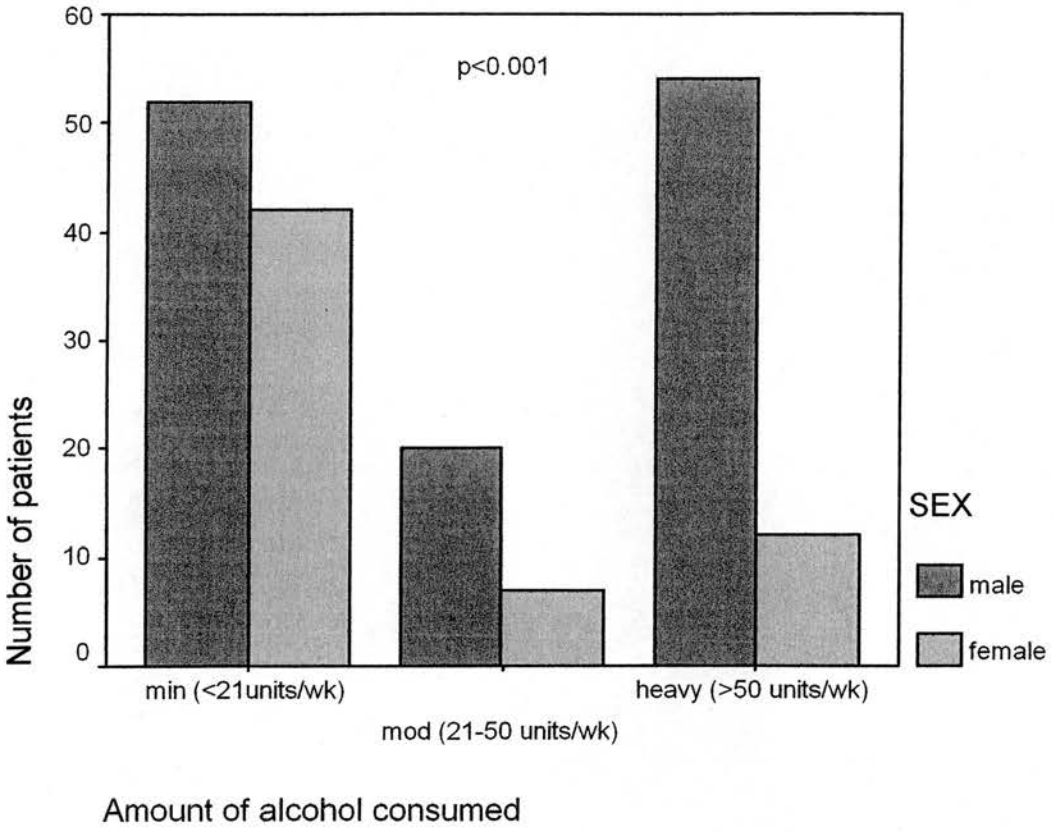


Figure 2.3

GENOTYPES

Genotyping of 75 patients was performed and the distribution of these genotypes is shown in figure 2.4. The most common genotype in those patients tested was 1 (54%).

DURATION OF INFECTION

Duration of infection is calculated as the time of infection to time of biopsy.The duration of infection was able to be calculated in 144 patients. The median duration of infection in our group of patients was 12 years.

HISTOLOGY

83.2% of our patients underwent liver biopsy and therefore have data on histology available. As described earlier the histological findings were recorded according to inflammation and fibrosis. This is shown in figure 2.5.

95 (55.9%) patients had mild inflammation, 66 (38.8%) had moderate inflammation and 7 (4.1 %) had severe inflammation. 2 patients had no inflammation.

With regards to fibrosis 37 (20.8%) patients had no fibrosis, 30 (16.9%) had portal spurring only, 56 (31.5%) had pericellular fibrosis, 20 (11.2%) had bridging fibrosis and 35 (20.8%) had cirrhosis.

The relationship of alcohol to fibrosis was also examined. There was a strong correlation of amount of alcohol consumed and degree of fibrosis on biopsy ($p<0.0001$). There was also a significant correlation between amount of alcohol consumed and degree of inflammation ($p=0.005$).

Genotypes of infected patients

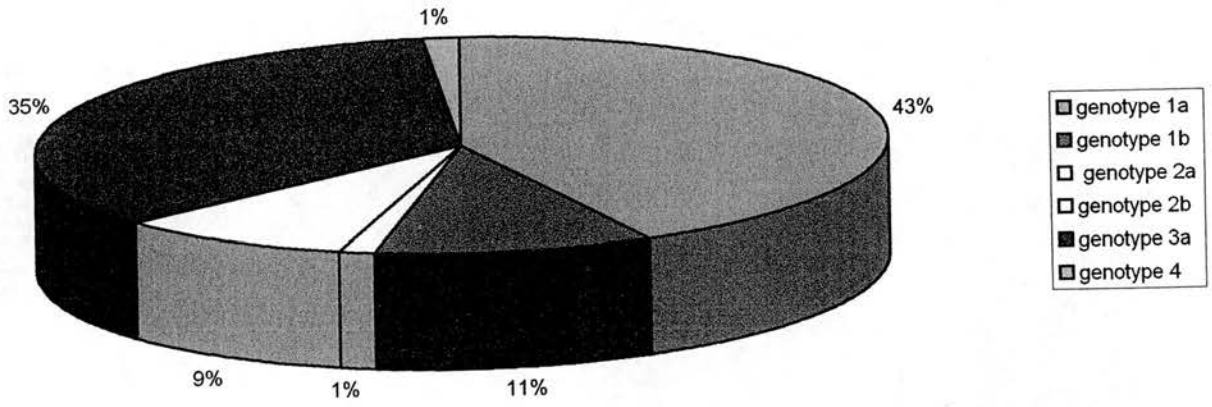
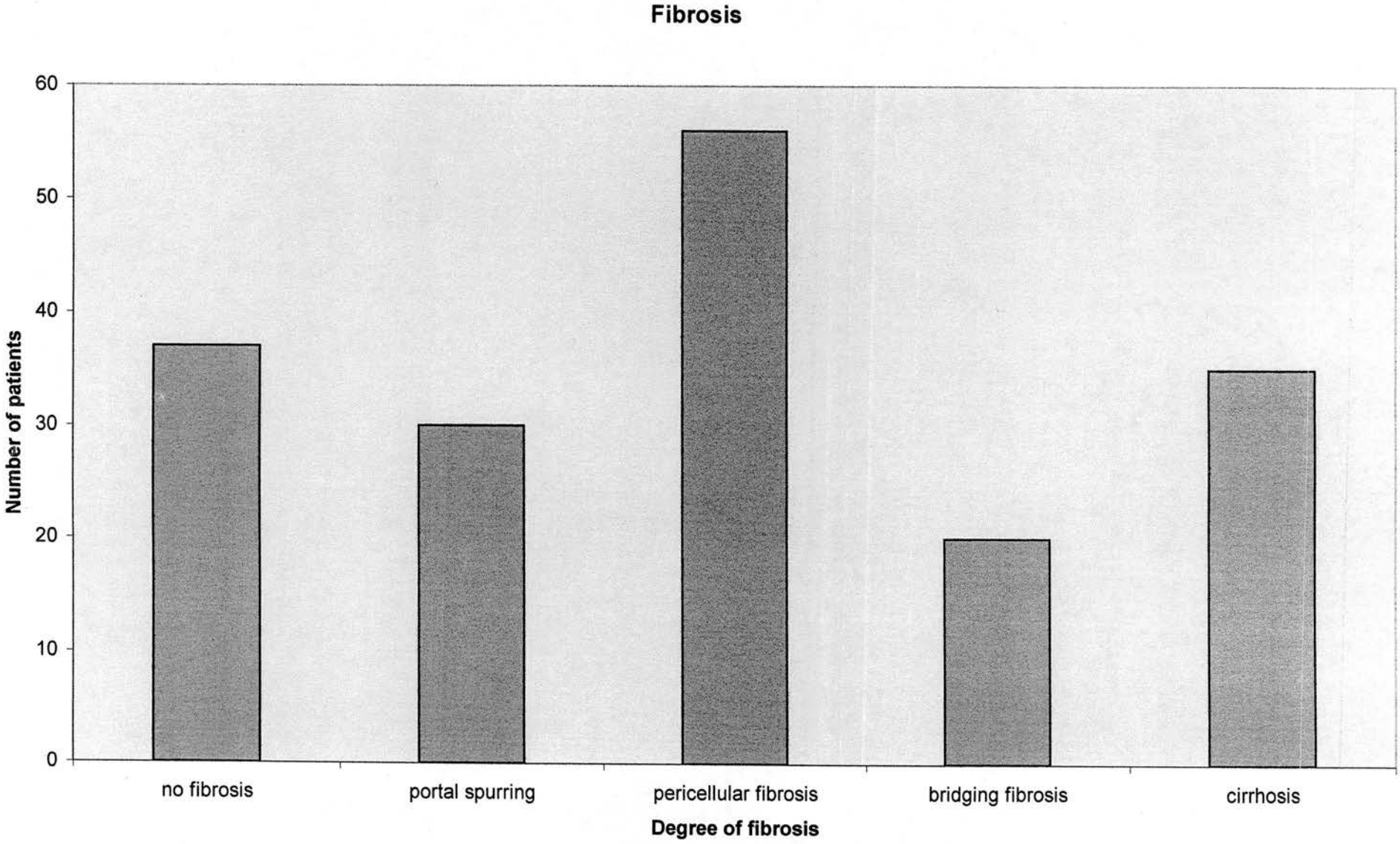
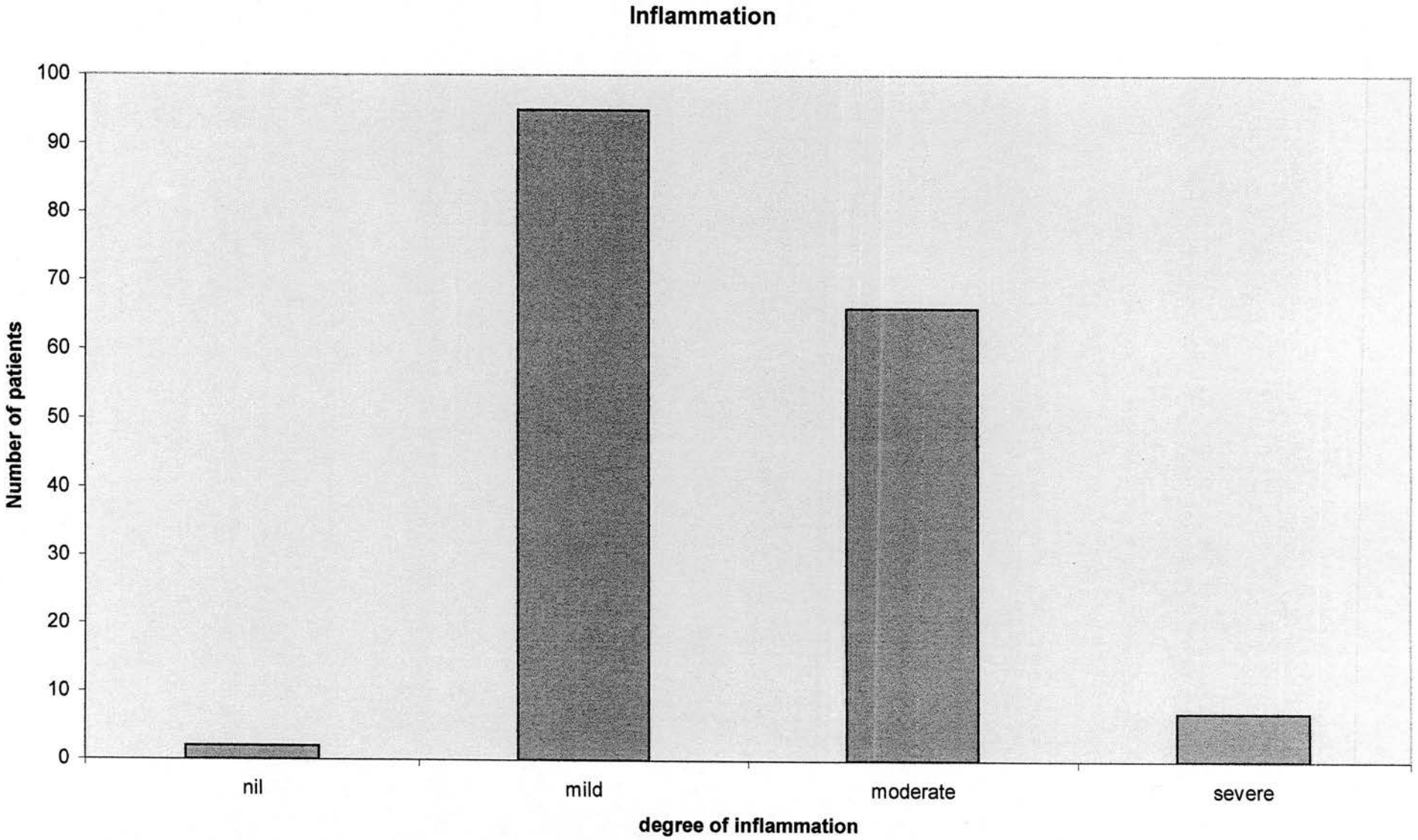


Figure 2.4


Fibrosis
60
50
40
30
20
10
0
Number of patients
no fibrosis
portal spurring
pericellular fibrosis
bridging fibrosis
cirrhosis
Degree of fibrosis


Inflammation
100
90
80
70
60
50
40
30
20
10
0
Number of patients
nil
mild
moderate
severe
degree of inflammation

Neither HIV status or hepatitis B status had a significant relationship with degree of fibrosis or inflammation. Genotype also had no significant effect on histological changes although the numbers were small (n=65).

Age at infection had no significant effect on degree of inflammation or degree of fibrosis.

In this cohort of patients there was no significant correlation between level of ALT and either degree of inflammation or amount of fibrosis.

Males were significantly more likely to have a greater degree of fibrosis than females ($p<0.05$). This however was not the case with respect to degree of inflammation. (Figure 2.6).

Regarding duration of infection there was a significant relationship between duration of infection and degree of fibrosis however there was no significant relationship with regards to level of inflammation.

Mode of acquisition of infection had no effect on either fibrosis or inflammation.

TREATMENT

65 patients underwent treatment with interferon-alpha.

Patients were treated using a standard treatment protocol. The treatment used was alpha interferon 3 megaunits three times per week for a maximum of 6 months.

Of these 2 patients stopped treatment early because of side effects.

The remainder completed 6 months of therapy. There were 4 sustained responders (defined as normalisation of ALT and absence of circulating RNA during treatment and maintained at six months after completion of treatment).

There were 23 non-responders (defined as no effect on ALT and HCV-RNA during or after treatment).

There were 36 relapsers (defined as normalisation of ALT and absence of circulating HCV-RNA during treatment followed by return of HCV-RNA following cessation of treatment. (Figure 2.7).

There was no significant difference in treatment responses according to mode of infection, genotype, or ALT prior to infection however there was a significant difference in response according to sex of the patient with females being significantly more likely to respond than males ($p<0.05$), however there was no significant difference in sustained response between males and females. (Figure 2.8).

OUTCOME

After 5 years follow up 22 patients had died

16 patients died from complications related to their liver disease, either hepatocellular carcinoma or decompensated liver disease. 6 patients died from other causes including suicide, lymphoma, stroke and death from violence.

4 further patients underwent OLT 2 for HCC and 2 for other complications relating to decompensated liver disease. One of the patients transplanted for HCC died subsequently

Degree of fibrosis according to sex

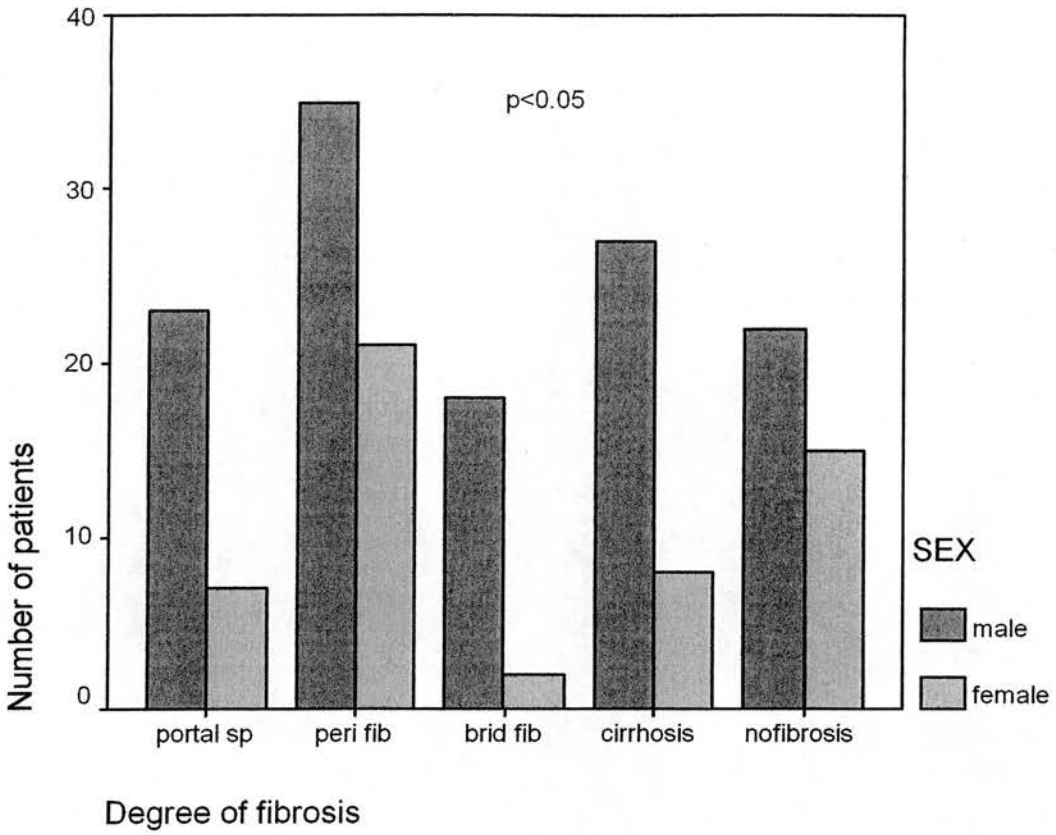
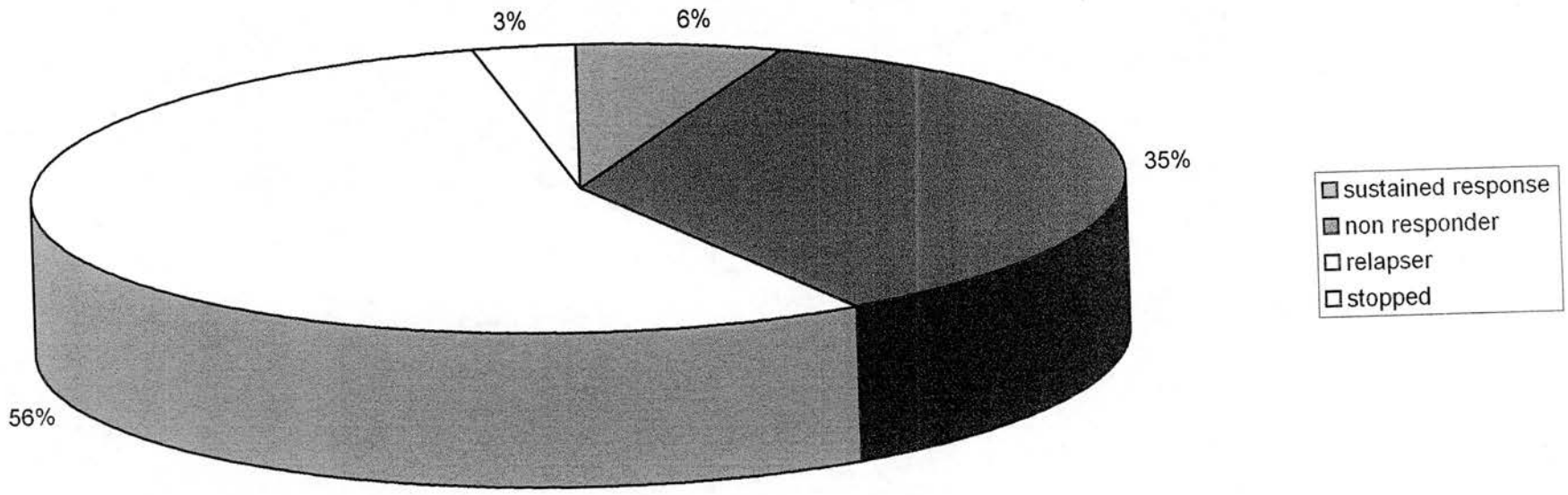


Figure 2.6

HCV cohort by response to interferon



Response according to treatment

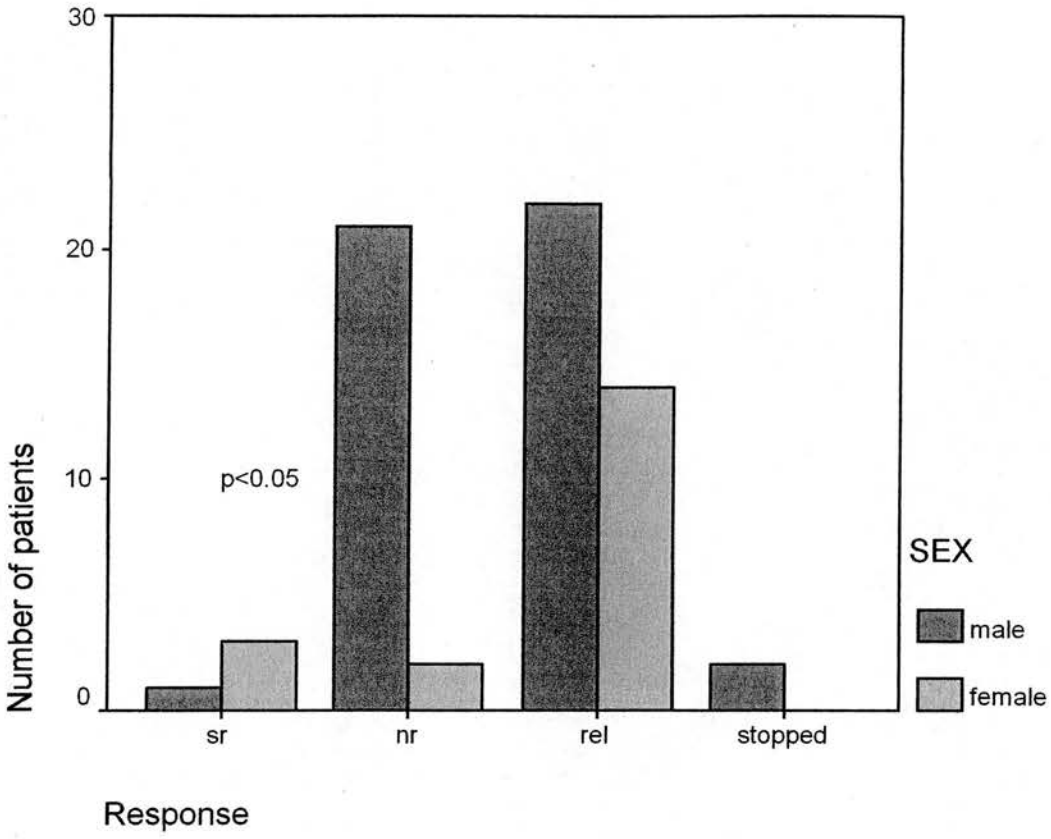


Figure 2.8

2.3.ii Progression of disease

Duration of infection was available in 144 patients. In this subgroup, progression to cirrhosis was calculated. The progression to cirrhosis according to different variables is illustrated in the following figures.

Progression according to sex

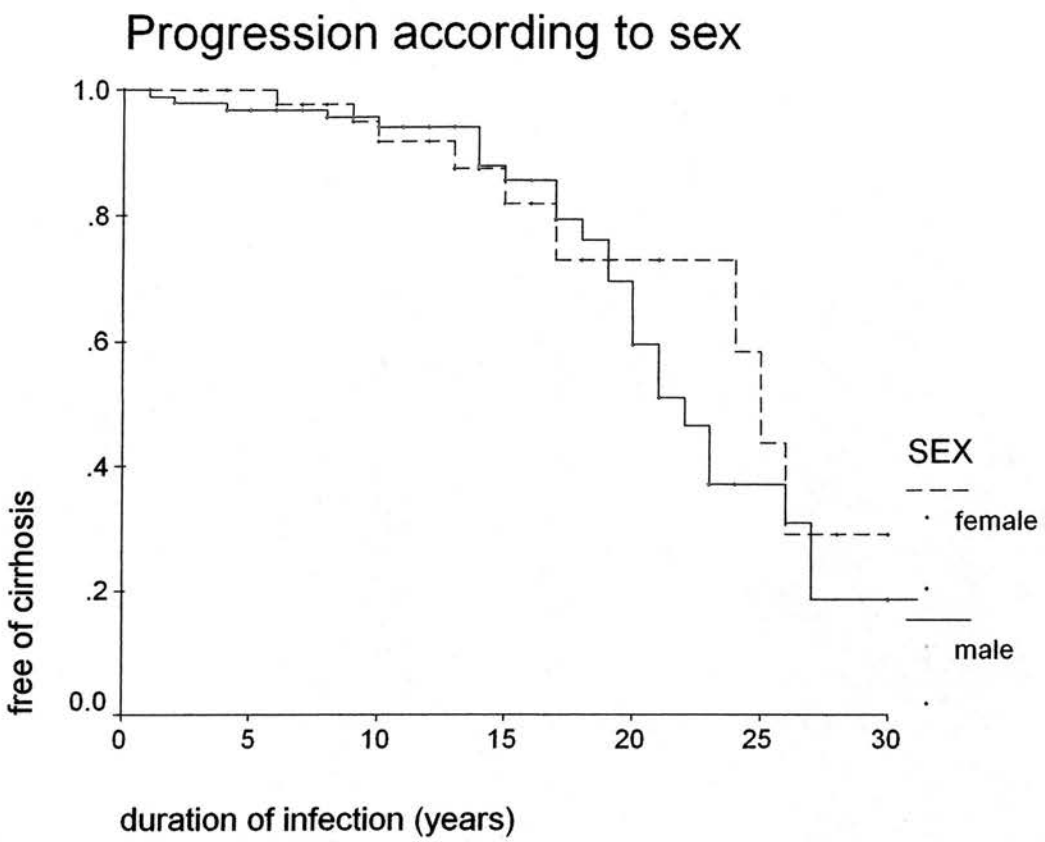
There was no significant difference in development of cirrhosis according to sex in our cohort of patients. (Figure 2.9).

Progression according to route of infection.

There was no significant difference in progression to cirrhosis in IVDU versus patients infected through blood transfusion. (Figure 2.10).

Progression according to alcohol consumption.

There was a statistically significant difference in progression to cirrhosis in patients who consumed greater than 50 units of alcohol per week when compared to those patients who consumed less than 50 units of alcohol per week. $P<0.005$. (Figure 2.11).

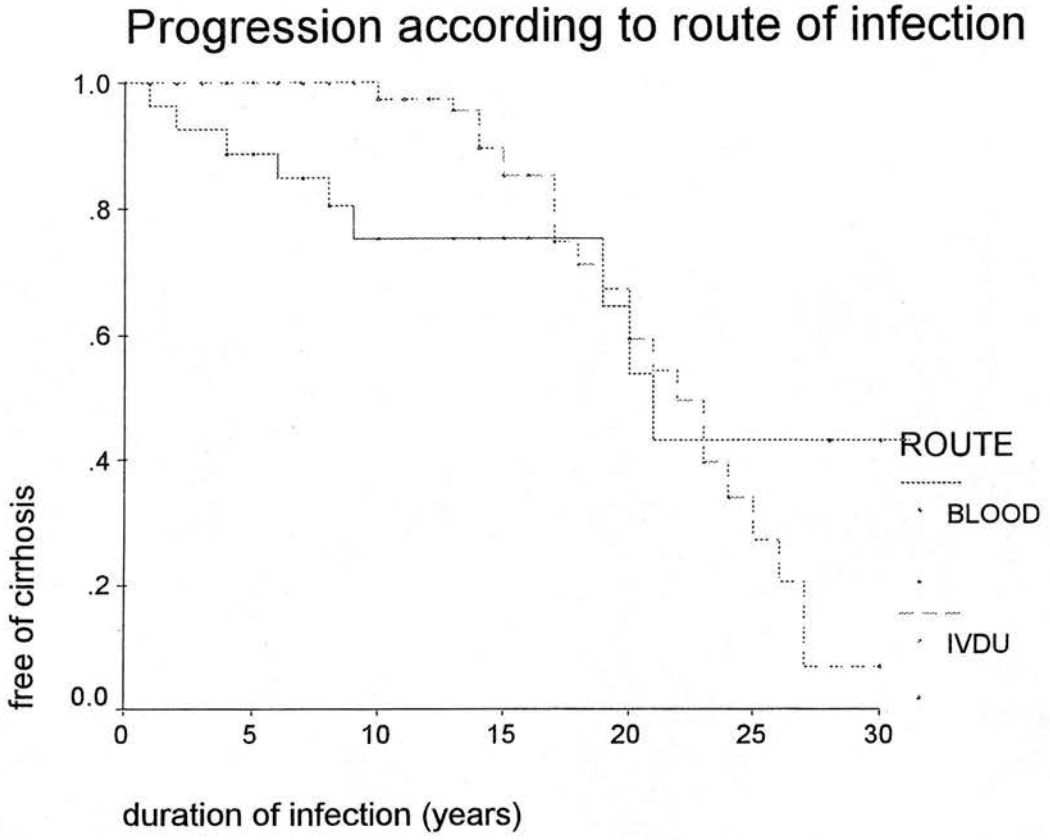


p=ns

Numbers at risk

time (years)	**0**	**5**	**10**	**15**	**20**	**25**	**30**
male	94	87	67	38	21	6	3
female	50	46	30	16	6	4	1

Figure 2.9

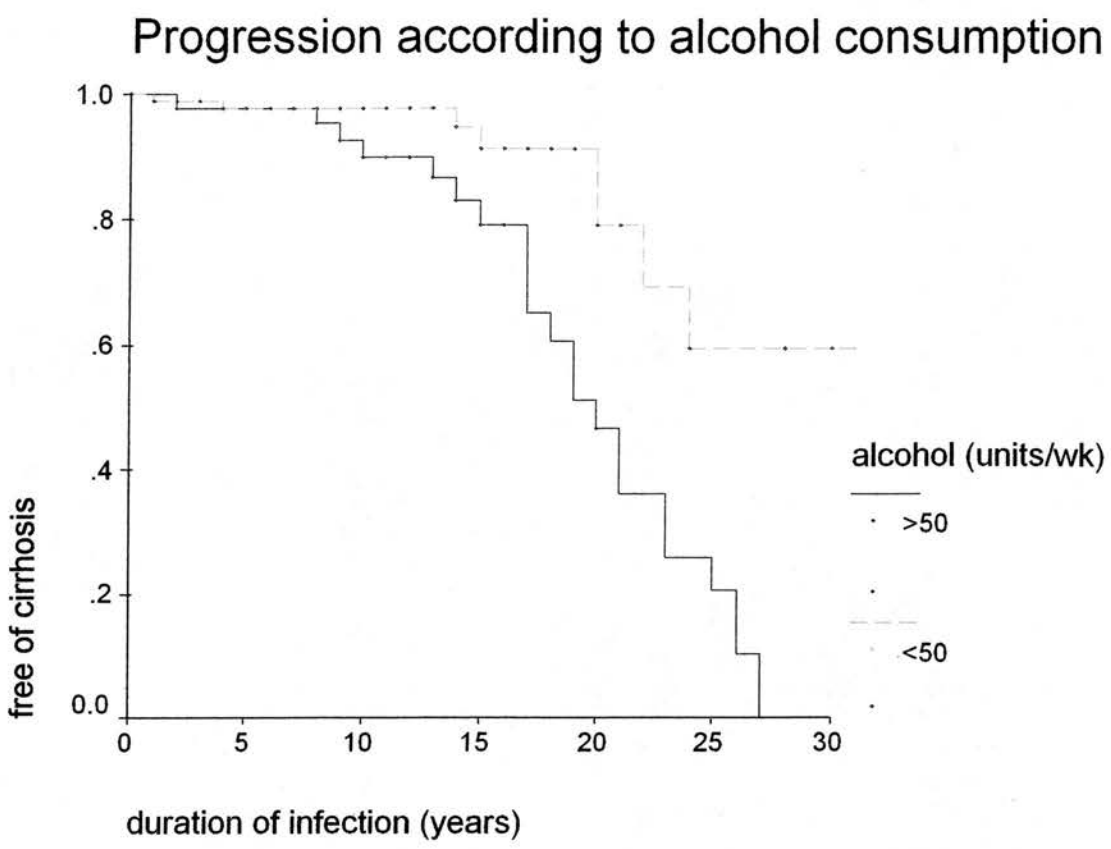


p=ns

Numbers at risk

Time (years)	**0**	**5**	**10**	**15**	**20**	**25**	**30**
Blood	28	23	14	9	6	4	3
IVDU	105	99	76	47	17	5	1

Figure 2.10



p<0.005

Numbers at risk

Time (years)	**0**	**5**	**10**	**15**	**20**	**25**	**30**
<50units/wk	87	80	56	29	15	5	4
>50units/wk	44	42	33	21	11	5	0

Figure 2.11

Progression according to hepatitis B status and HIV status

There was no significant difference in rate of progression to cirrhosis in patients infected either with hepatitis B or HIV infection. (Figure 2.12).

Progression according to age at infection

There was a significant difference in development of cirrhosis according to age with patients infected after the age of 40 having a faster progression to cirrhosis $p<0.0001$. (Figure 2.13).

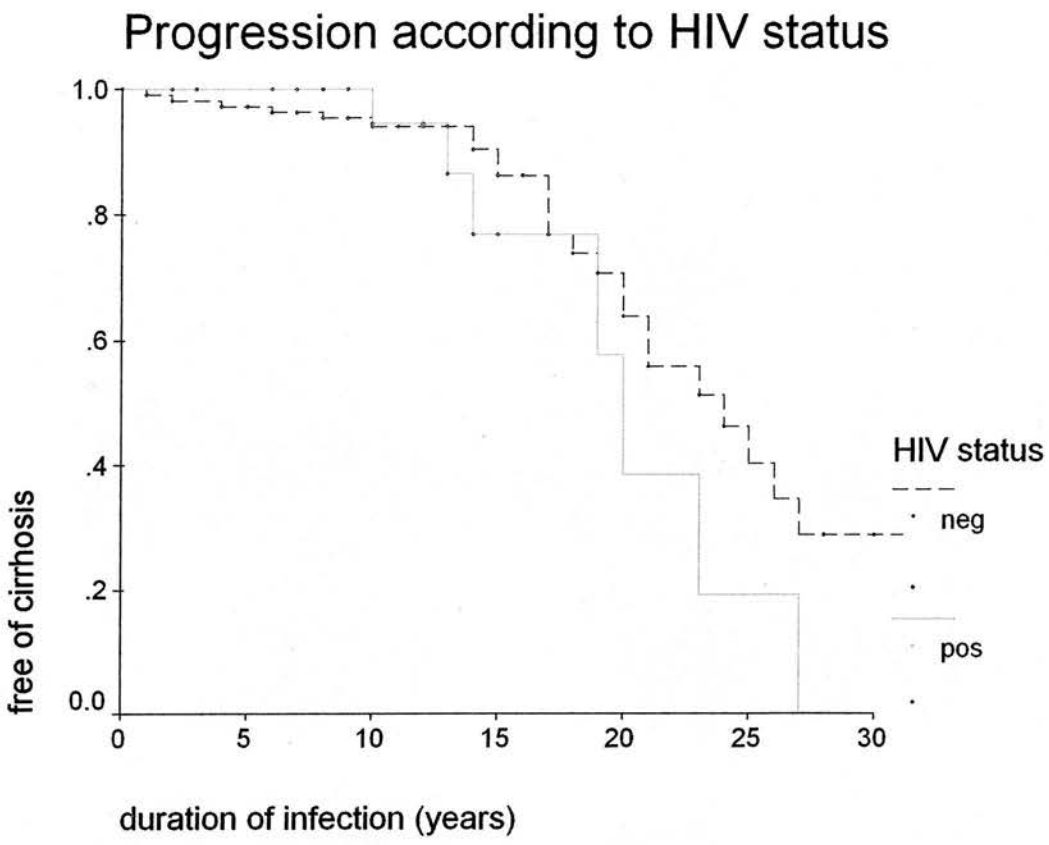
Progression according to duration of infection

The overall progression to cirrhosis with time is demonstrated in figure 2.14.

The median time to development of cirrhosis in the overall cohort was calculated as 23 years (95% confidence interval 20.62 to 25.38)

Cox regression analysis

The independent risk factors identified for progression to cirrhosis were an older age at infection (>40 years) and a previous alcohol consumption of greater than 50 units/week for at least 5 years.

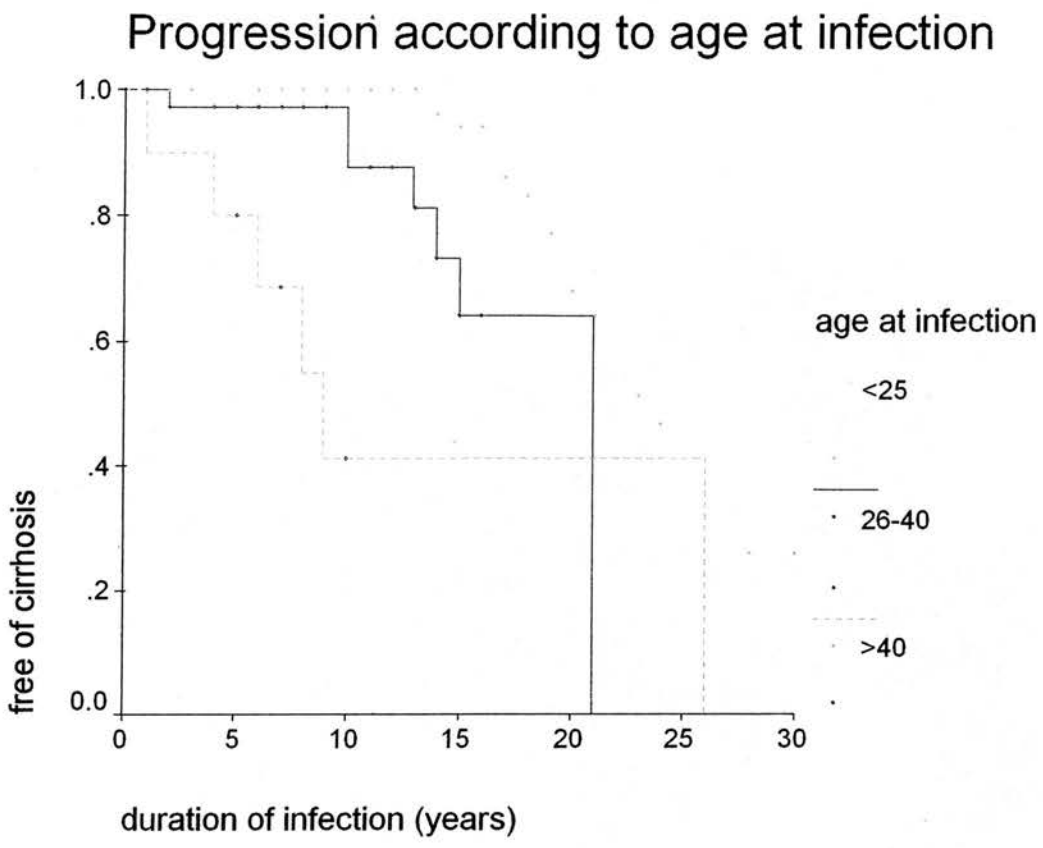


p=ns

Numbers at risk

Time (years)	**0**	**5**	**10**	**15**	**20**	**25**	**30**
HIV pos	26	24	18	6	3	1	0
HIV neg	113	104	75	44	21	8	4

Figure 2.12

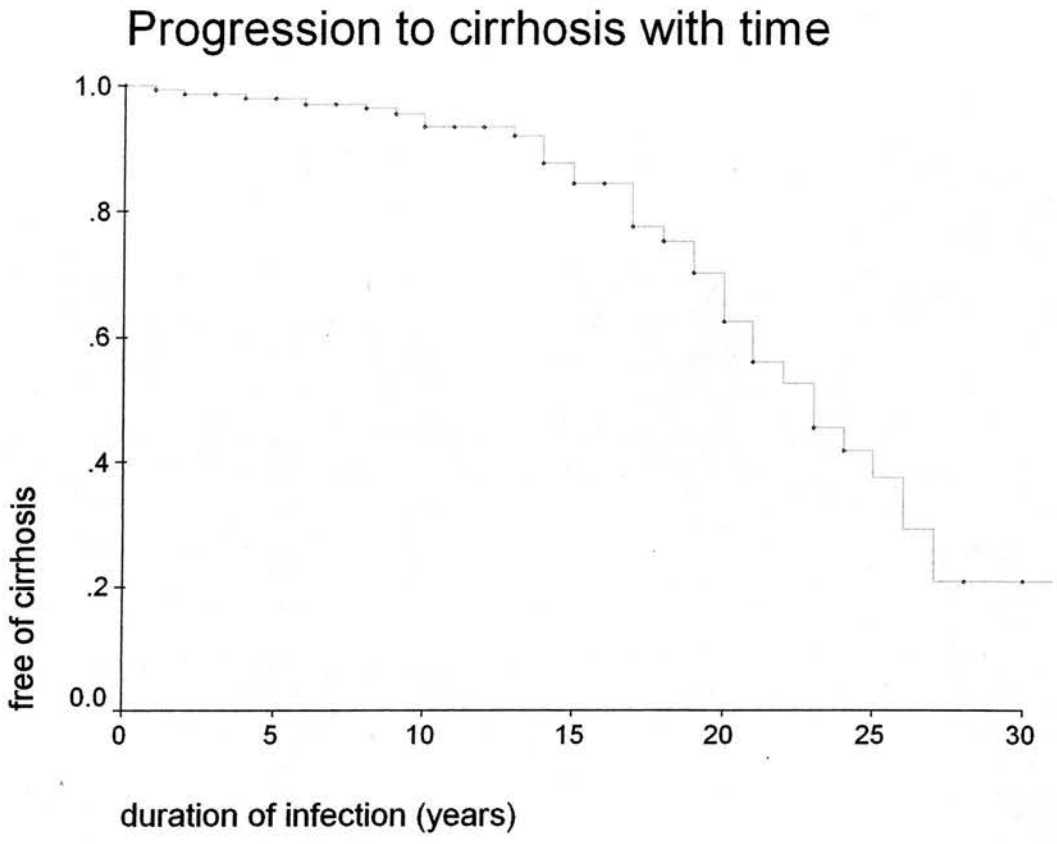


p<0.0001

Numbers at risk

Time (years)	**0**	**5**	**10**	**15**	**20**	**5**	**30**
<25 years	95	92	74	45	25	9	4
26-40 years	38	33	20	8	1	0	0
>40 years	11	8	3	1	1	1	0

Figure 2.13



Numbers at risk

Time (years)	**0**	**5**	**10**	**15**	**20**	**25**	**30**
	144	133	97	54	27	10	4

Figure 2.14

2.4 Discussion

These data summarise the demographic characteristics of the cohort of patients infected with chronic HCV in South East Scotland. The factors relevant to progression to cirrhosis are also examined.

In this geographical area there is a fairly large number of injecting drug users relative to many other areas in the UK and this is demonstrated by the large percentage of patients with IVDU as their mode of transmission of infection (64%). The low mean age of infection is largely related to this fact as it is known that the majority of IVDU begin injecting drugs in their late teenage years and early twenties. Co infection with other parenterally transmitted viruses was not uncommon with 16.7% of patients being coinfected with HIV and 27.7% having evidence of previous hepatitis B infection. There were relatively few patients with active hepatitis B infection presumably due to the much lower rate of chronicity of hepatitis B virus infection compared to HCV infection.

The alcohol consumption in our cohort of patients was high in common with many other studies and the role of alcohol as an important cofactor in the progression of chronic HCV infection to cirrhosis will be discussed later. The relatively greater consumption of alcohol by males was also noted.

We have data on liver histology on a significant number of the cohort (83.2%).

It is now apparent that while the degree of inflammation can fluctuate over time, the development and progression of fibrosis is thought to be largely irreversible and that it is this progression of fibrosis that results in the development of cirrhosis and its complications e.g. varices, ascites and hepatocellular carcinoma. However the

association between fibrosis progression and the necroinflammatory scores on liver biopsy is controversial. It has been shown that on a single liver biopsy there is little or no correlation between severity of necroinflammatory activity and degree of fibrosis. (Poynard et al 1997, Poynard et al 2001). However in several recent studies an association has been shown between the degree of necroinflammatory activity and subsequent progression of fibrosis. (Ghany et al 2000, Alberti et al 2001). This is in keeping with the findings in the cohort that more severe inflammation was associated with more progressive fibrosis.

In the group of biopsied patients 20.8% had cirrhosis at the time of biopsy. The median time to development of cirrhosis was 23 years. The majority of patients had either mild or moderate inflammation (94.7%).

There was a strong relationship between alcohol consumption and degree of fibrosis. This is in agreement with many other studies. (Poynard et al 1997, Roudot-Thoraval 1997, Verbaan et al 1998). In addition those with heavier alcohol consumption had significantly more inflammation than those who consumed lower amounts of alcohol. The significance of this is less clear. Genotype, hepatitis B status and HIV status had no significant effect on either amount of fibrosis or inflammation. The results relating to genotype may be in part due to the small numbers of our patients who had been genotyped. However other groups have concurred with this finding (Poynard et al 1997, Verbaan et al 1998).

Age at infection had no significant effect on fibrosis or inflammation scores although, in common with other studies, when progression to cirrhosis according to age at infection was examined there was a significantly faster development to cirrhosis in

those infected over 40 years of age in (figure 2.9). This is in agreement with other studies. (Poynard 1997, Freeman et al 2001, Poynard et al 2001).

There was no correlation between ALT and degree of inflammation or fibrosis in our cohort. It is well recognised that ALT fluctuates during the course of chronic HCV infection (Alter et al 1992). Several studies have been performed assessing patients with persistently normal ALT over several months and have confirmed that ALT levels are a poor guide to liver histology with some patients with relatively advanced histological changes having normal transaminase levels and vice versa. (Stanley et al 1996, Healy et al 1995, Naito et al 1994, Bruno et al 1994). However some longitudinal studies have demonstrated that ALT titre at time of initial biopsy can be correlated with progression of fibrosis (Ghany et al 2000). Marcellin et al showed that patients with a mean serum ALT level of twice the normal range had a greater risk of progression of fibrosis on a repeat liver biopsy 3 years later. (Marcellin et al 2001). We have not performed sequential biopsies in our patients and therefore cannot confirm this finding in our population.

With respect to sex of patients there was a significant relationship between amount of fibrosis and male sex although the role of alcohol as an additional factor needs to be examined. Poynard et al in their study had similar findings with regard to sex and other authors have reported a similar relationship. (Poynard et al 1997).

Relatively few patients had undergone treatment with alpha-interferon. The definitions of response are shown in table 1.2 .The sustained response rate was 6%, the non-responder rate 35% and the relapser rate was 56%. 2 patients (3%) stopped treatment because of side effects. Over all the sustained response rate with 6 months treatment with 3 megaunits thrice weekly was disappointing. Our sustained reponse rates were

obviously lower than many of the trials previously reported. This may be due to different methods of patient selection, use of dosage regimens that are now known to be suboptimal and perhaps differing compliance rates amongst people attending our clinic and those taking part in clinical trials. Interferon monotherapy has now largely been superseded by combination therapy with ribavirin and this has resulted in a significant improvement in sustained reponse rates in our patients.

Our patients now receive 6-12 months combination therapy with ribavirin and interferon alpha and the results so far are much improved with respect to sustained response.

The natural history and progression of chronic hepatitis C is complex. There are many cross sectional and retrospective studies in the literature examining the natural history of the disease and progression to cirrhosis. Of interest also are those factors which may accelerate, promote, prevent or delay progression to cirrhosis.

In this population we found no significant effect of gender on progression to cirrhosis. This is in contrast to several other studies where male sex has been associated with a more rapid progression of liver disease. (Bissell et al 1999, Poynard et al 2001, Freeman et al 2001). It is interesting that we found higher scores in fibrosis in male patients and it may simply be that duration of infection is longer in our group of male patients.

It does now seem clear that age at time of infection has a significant effect on progression of fibrosis in patients with chronic HCV infection. In our cohort there was a highly significant relationship between age at infection and time to cirrhosis with those infected at over 40 years having a significantly reduced time to development of cirrhosis when compared with younger patients. Other groups report

similar findings.(Poynard et al 1997, Freeman et al 2001, Poynard et al 2001). There are various hypotheses as to why this occurs. These include impaired host defence mechanisms, increased fibrogenesis or decreased fibrinolysis on older people.

The route of infection had no significant effect on progression to cirrhosis.

This concurs with several other studies although there are a few reports of accelerated progression in patients infected by blood transfusion. (Alter et al 1992, Roudot-Thoraval 1997). Overall however it is now thought unlikely that route of infection has any significant effect on development of cirrhosis.

Excessive alcohol consumption is now known to result in more rapid progression of disease in chronic HCV infection. In the Edinburgh cohort those who consumed greater than 50 units of alcohol per week developed cirrhosis significantly earlier than those who consumed less than 50 units of alcohol per week. There were also significant differences in duration to cirrhosis in those who consumed either minimal (<21 units per week) or moderate (21-50 units per week) amounts of alcohol when compared to those who had heavy (>50 units of per week) alcohol consumption. There was however no significant difference in development of cirrhosis when those drinking minimal amounts of alcohol were compared to moderate alcohol consumers. There are many other studies reporting broadly similar findings. (Table 2.1).

author	Alcohol units	Findings	P value
Roudot-Thoraval 1997	Heavy >5(women) and 6(men) drinks per day for more than one year	Cirrhosis more common in those who drank excessively	p<0.001
Serfaty 1997	<30g/day, 30-80g/day, >80g/day	Those consuming >30 g day at increased risk of developing cirrhosis	p<0.05
Poynard 1997	None, <50g, ≥50g of alcohol per day	Mean stage of fibrosis higher in those who drank more than 50g alcohol per day	p<0.001
Corrao 1998	LDAI (lifetime daily alcohol intake calculated)	Dose-effect relationship between LDAI and risk of liver cirrhosis. Additive risk for <50g/day and multiplicative risk for consumption >125g/day	
Niederau 1998	<80g/day, ≥80g/day	Survival reduced in patients who consumed ≥80g of alcohol per day	Risk ratio 1.6 (0.7-3.4)
Wiley 1998	Excessive >40g/day women and >60g/day men for > 5 years	Rate of progression to cirrhosis greater in excessive consumers of alcohol	p<0.05
Pessione F 1998	SRAC(self reported alcohol consumption)	Significant correlation between fibrosis and SRAC	p=0.006
Verbaan 1998	<80g/day, ≥80g/day for at least five years	Excess alcohol was associated with progression to cirrhosis	p=0.007

Author	Alcohol units	Findings	P value
Bellentani 1999	<30g/day, >30g/day	Consumption of >30g/day associated with 3X higher risk of cirrhosis	p<0.01
Thomas 2000	grammes/week	Relative risk of end-stage liver disease was 3.6 (1.73-7.52) for those drinking >260g/week of alcohol	
Harris 2001	g/day	Consumption of >80g/day was associated with 4X-increased risk of cirrhosis.	OR 4.0 (2.1-7.7)
Westin 2002	<40g/day	Higher consumption of alcohol and higher drinking frequency associated with more progressive fibrosis.	P=0.03
Harris 2002	None, <30g/day, >30g/day	Increased risk of fibrosis and decreased survival in those consuming >30g/day	RR 1.97 for fibrosis and 1.18 for survival

Table 2.1:summary of studies reporting effects of alcohol on patients with chronic HCV infection

Although there are many studies that examine the risk of heavy alcohol use what is less clear is whether moderate or even low alcohol intake is associated with a higher rate of progression when compared to those that are completely abstinent from alcohol. We have not shown any difference in progression between those drinking <21 units per week versus those drinking 21-40 units/week. However we did not separate those that were drinking no alcohol at all from the minimal alcohol group and it would be interesting to compare progression to cirrhosis in patients entirely abstinent from alcohol and those that drink either minimal or moderate amounts.

Coinfection with hepatitis B infection is relatively common in patients with HCV infection. Several previous studies have examined the role of viral interference in dual HBV and HCV infection. The results have been conflicting with some suggesting that HCV possesses the strongest suppressant effect (Pontisso et al 1996, Crespo et al 1997) and other suggesting that HBV is more dominant (Ohkawa et al 1995, Wang et al 1999). More recently a study from Taiwan has examined the role of HBV in acute HCV infection. (Chu et al 2002). This group found that the incidence of persistent HCV infection correlated with the underlying status of HBV replication. The incidence of persistent HCV infection in chronic HbsAg carriers without active replication was similar to that in non hepatitis B carrier patients. However acute HCV infection in chronic HbsAg carriers with active replication rarely progressed to chronic infection suggesting that the presence of active HBV-DNA replication could inhibit the persistence of HCV infection and also antibody responses to HCV. A further recent study from Spain has examined viral replicative interference. They found that HBV-DNA and HCV-RNA levels were lower in co-infections than in single infections. How these studies relate to progression of disease is less clear

however because there are several studies that demonstrate that HBV appears to have an additive effect on the rate of fibrosis progression. (Tsai et al 1996, Pontisso et al 1998, Cacciola et al 1999). There was no significant effect on progression in our cohort however the small numbers with active infection make our results difficult to interpret.

Similarly with HIV infection co infection is not uncommon. There are now many studies that have demonstrated that coinfection with HIV is associated with a more rapid progression of HCV. (Benhamou et al 1999, Lesens et al 1999, Ragni et al 2001, Di Martino et al 2001, Thomas et al 2002). Again our numbers are small making it difficult to draw firm conclusions on our cohort.

Finally the independent variables that were significantly associated with development of cirrhosis were demonstrated in the Cox regression model as older age at infection and heavy alcohol consumption.

In summary this chapter describes the Edinburgh HCV cohort and examines factors that are important in progression to cirrhosis. With recognition of the factors associated with progression of disease, screening for complications and the improving results with combination therapy the outcome of this relatively common disease will hopefully improve.

CHAPTER 3

HLA STATUS AND PROGRESSION AND SEVERITY OF HEPATITIS C INFECTION.

3.1 Introduction

Infection with the hepatitis C virus (HCV) is the cause of 90% of the cases of non-A, non-B hepatitis and most of the cases of post transfusion hepatitis. The natural history of the disease is complex with a wide spectrum of disease; Less than 10% of patients develop acute illness, whilst at least 80% of infected patients develop chronic liver disease ranging from minimal hepatitis to cirrhosis and, in a significant number, hepatocellular carcinoma (Alter et al 1992). Factors postulated to be important in the development and progression of chronic liver disease in HCV infection have been discussed in chapter 1 and include **viral factors** (e.g. high virus level (Hagawara et al 1993, Booth et al 1995, Lau et al 1993), HCV genotype(Booth et al 1995, Dusheiko et al 1994) and diverse quasispecies (Honda et al 1996, Farci et al 1996)), **host factors** (e.g. age (Poynard et al 1997), mode of transmission (Alter et al 1992, Gordon et al 1993, Roudot Thoraval et al 1997); duration of infection (Kiyosawa et al 1990)) and **cofactors** (e.g. Hepatitis B virus infection (Alberti et al 1994, Fong et al 1990, Benvegnu et al 1994), Human Immunodeficiency Virus infection (Martin et al 1989, Eyster et al 1993)and heavy alcohol intake (Poynard et al 1997,Roudot-Thoraval et al 1997, Seef et al 1992)).

Consequently, the lack of efficient immune responses are almost certainly also involved in the development and progression of chronic hepatitis C infection. It is likely that differences in the immunogenetic background of infected patients might in part account for the wide variation in disease progression observed amongst diseased individuals. Previously, it has been demonstrated that polymorphisms of immune regulatory genes on HLA class I and II molecules can influence the host's ability to present or react to viral antigens.

Studies from different geographical areas have supported the role of HLA-DR molecules, in particular, in the pathogenesis of chronic hepatitis C infection. While a protective role for HLA-DR5 (Zavaglia et al 1996) and DR2 (Congia et al 1996) has been reported for North European populations, the presence of HLA DR5 has been associated with a benign course of HCV induced liver disease in Caucasians (Peano et al 1994), while a similar role has been described for HLA-DR13 in Japanese HCV carriers (Kuzushita et al 1996).

In this study, we investigated the distribution of common HLA class I and II antigens in a Scottish population with chronic HCV according to the severity and persistence of infection.

3.2 Subjects and methods

3.2.i subjects

patients

Seventy-one patients (41 male and 30 female) were recruited for the study. All were attending our hepatitis C clinic. Each patient was HCV-Ab positive and positive for HCV-RNA by RT-PCR, HBsAg negative; no other cause had been identified for their liver disease. Patients underwent diagnostic liver biopsy; which was examined by a pathologist experienced in interpreting HCV associated liver disease and divided into chronic hepatitis or cirrhosis. All patients were Caucasian and of North European extraction to avoid ethnic HLA variability.

controls

The control population consisted of a cohort of 264 (132 male, 132 female) age matched Scottish Caucasians described previously (Jazwinska et al 1987). Most normal control data on HLA antigen frequency are derived from blood donors or laboratory personnel. This control group were derived from healthy new parents recruited at the Simpson Memorial Maternity Pavilion. The major bias could be that the population is taken from a relatively narrow age range. To counteract this possibility the researchers examined the neonatal antigen frequencies which were not found to be significantly different from the parent group. This therefore excludes the unlikely possibility of HLA associations with death before reproductive age and infertility. It was therefore felt that this control group were an appropriate group to use to compare with our HCV infected patients.

All patients recruited to the study gave informed consent and ethical approval was obtained for the study.

3.2.ii methods

Complete physical examination was performed in each case. Standard tests of liver function and inflammation were obtained and repeated on at least 3 separate clinic visits.

Each patient was typed for HLA class A, B and DR using a two-stage complement dependent microlymphocytotoxicity technique as previously described (Terasaki 1980). (The majority of this work was performed by Mary McColl and Lorraine Deane in the tissue typing laboratory, RIE)

Patients were tested for the following antigens common in the Scottish population.

A1,A2,A3,A11,A24; B7,B8,B35,B44; DR1,DR2,DR3,DR4,DR5,DR6,DR7

3.2.iii statistics

Comparison between HLA allele frequencies was performed by Chi-squared analysis. P values were corrected (Pc) for the number of antigens used according to the method of Bonferroni. Values of $Pc<0.05$ were taken as statistically significant.

3.3 Results

71 liver biopsy results were available. 17 patients (24%) had biopsy proven cirrhosis and 54 (76%) had chronic hepatitis including portal inflammation, lobulitis and fibrosis. Of the patients with chronic hepatitis 13/54 (24%) had a normal ALT value on 3 or more consecutive occasions. The remainder (41) had an elevated level.

The distribution of HLA class A, B and DR frequencies in the study population of 71 patients and the population of 264 normal controls is shown in tables 3.1-3.3.

Table 3.1 shows the frequencies in the healthy population versus the HCV infected patients. Table 3.2 illustrates the HLA frequencies in patients with chronic hepatitis and a normal ALT value versus those with chronic hepatitis with an elevated ALT level. Table 3.3 reports the HLA frequencies in patients with chronic hepatitis on liver biopsy versus those with biopsy proven cirrhosis.

Healthy versus HCV infected patients

We found that the frequency of DR5 was significantly higher in the normal population (12%) compared with HCV infected patients (0%) ($Pc=0.006$) suggesting that the presence of the DR5 allele protects against the development of chronic disease.

Normal ALT versus raised ALT

DR6 was significantly associated with persistently normal transaminases ($Pc<0.05$) while B7 and DR7 were associated with abnormal transaminases ($Pc<0.0016$).

Chronic Hepatitis versus cirrhosis

HLA B35 occurred significantly more frequently in those patients with chronic hepatitis (13%) when compared with those with cirrhosis (0%) ($Pc<0.005$), suggesting that presence of the B35 allele protects against the development of cirrhosis in patients infected with HCV.

HLA LOCUS	CONTROLS %	n	CHRONIC HCV %	n	Pc
A1	37	97	32	23	NS
A2	50	131	53	38	NS
A3	31	81	21	15	NS
A11	13	33	4	3	NS
A24	14	37	18	13	NS
B7	33	86	38	27	NS
B8	31	80	19	14	NS
B35	12	32	10	7	NS
B44	28	73	36	25	NS
DR1	10	27	14	10	NS
DR2	29	77	34	24	NS
DR3	31	83	29	21	NS
DR4	25	66	26	19	NS
DR5	**12**	**33**	**0**	**0**	**0.006**
DR6	35	93	25	18	NS
DR7	33	86	34	24	NS

Table 3.1: Frequencies of HLA antigens in normal versus HCV infected patients

HLA LOCUS	NORMAL ALT % n		RAISED ALT % n		Pc
A1	46	6	32	13	NS
A2	66	8	54	22	NS
A3	7	1	27	11	NS
A11	0	0	2	1	NS
A24	15	2	21	9	NS
B7	**15**	**2**	**41**	**17**	**<0.0016**
B8	15	2	24	10	NS
B35	15	2	12	5	NS
B44	30	4	32	13	NS
DR1	7	1	17	7	NS
DR2	41	5	32	13	NS
DR3	7	1	34	14	NS
DR4	30	4	24	10	NS
DR6	**38**	**5**	**19**	**8**	**0.048**
DR7	**15**	**2**	**39**	**16**	**<0.0016**

Table3.2: Frequencies of HLA antigens in patients with normal ALT versus abnormal ALT

HLA LOCUS	HEPATITIS		CIRRHOSIS		Pc
	%	n	%	n	
A1	35	19	23	4	NS
A2	55	30	47	8	NS
A3	22	12	23	4	NS
A11	1	1	11	2	NS
A24	20	11	11	2	NS
B7	35	19	47	8	NS
B8	24	12	11	2	NS
B35	**13**	**7**	**0**	**0**	**0.0032**
B44	31	17	41	8	NS
DR1	15	8	11	2	NS
DR2	33	18	35	6	NS
DR3	27	15	35	6	NS
DR4	26	14	29	5	NS
DR6	24	13	29	5	NS
DR7	33	18	35	6	NS

Table 3.3: Frequencies of HLA antigens in chronic hepatitis versus cirrhosis

3.4 Discussion

In this study, we have examined HLA class A, B and DR antigens in 71 Scottish, HCV infected individuals and compared the results with a well-defined age and sex-matched control population. The results suggested that certain antigens HLA DR5, DR6 and B35 may protect against the development of chronic disease, significant inflammation and hepatic cirrhosis respectively. Conversely, other antigens, HLA B7 and DR7 may predispose to the development of hepatic inflammation.

The role of host HLA antigens has been examined in other liver diseases, autoimmune chronic active hepatitis (AICAH), primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC) and chronic hepatitis B infection. DR3 and DR4 contribute to susceptibility to AICAH (Donaldson et al 1991a, Doherty et al 1994, Seki et al 1992) whilst in PBC, DR8 has a strong association with disease development in Japanese patients (Maeda et al 1992) but only a weak association in European and North American populations (Begovitch et al 1994, Gores et al 1997). In PSC, DR3 may predispose to the development of disease (Donaldson et al 1991b) The associations in hepatitis B infection are less convincing with several groups finding no association (Zavaglia et al 1996, Czaja et al 1996, Verdon et al 1994) but two groups reporting positive associations (Almarri et al 1994, Thursz et al 1995).

There have now been several studies examining the role of host HLA status in chronic HCV infection. There has been and still is significant interest in whether certain HLA antigens or alleles predispose to or, more importantly, protect against the development of chronic HCV infection. In the first of two Italian studies an HLA DR2 subtype was identified as protective against the development of chronic HCV

infection in Sardinian multiply-transfused patients (Congia et al 1996). In the second study the DR5 locus was significantly more common in healthy controls versus patients with chronic hepatitis implying it is a protective factor against the development of chronic disease (Zavaglia et al 1996). These data fully concur with the findings of this study where 12% of our healthy controls had the HLA-DR5 allele versus none of our HCV infected patients.

The gold standard for HLA typing is now recognised to be DNA typing. When carrying out this study DNA typing was not routine in our laboratory and there were various technical problems with DNA extraction. For these reasons this study was conducted using only the microlymphocytotoxicity technique described above. Other authors subsequently have investigated the distribution of HLA subtypes and alleles using the polymerase chain reaction and sequence specific primers. Tibbs and his group were the first to demonstrate the importance of host HLA DQ alleles. They concluded that DQB1*0302 and DQB1*03 were associated with protection from the development of chronic HCV infection in their Northern European cohort (Tibbs et al 1996). An Italian group reported that the haplotype DRB1*1104,DQA1*0501,DQB1*0301 protects against the development of chronic disease and the transdimer DQA1*0201 DQB1*0201 predisposes to the development of chronic and persistent HCV infection (Zavaglia et al 1998). These studies all suggest a role for the HLA class II antigens DR and DQ in the protection from or susceptibility to the development of chronic disease in patients infected with HCV.

The role of host HLA status and disease inflammatory activity has also been examined. DR5 was associated with a more benign course of disease in North American patients (Peano et al 1994) while a similar role has been described for DR13

in Japanese HCV carriers (Kuzushita et al 1996). In our cohort, HLA DR6 was significantly more common in patients with persistently normal ALT compared with the population with raised transaminase levels thus perhaps affording protection against severe disease. In all three studies the populations were either symptom free individuals with persistently normal ALT or with chronic hepatitis on liver biopsy and abnormal transaminase levels. However the value of ALT titre in predicting degree of inflammation is controversial (as discussed in chapter 2). More recently evidence has emerged that suggests that a persistently normal ALT may be associated with a slower rate of progression to cirrhosis. (Mathurin et al 1998, Freeman et al 2001). This may therefore suggest that DR6 in our population is associated with a more benign course of disease i.e. slower progression to cirrhosis and that patients with the B7 and DR7 antigens have a more rapid progression of disease to cirrhosis.

Finally in our study, HLA B35 antigen was protective against the development of cirrhosis. Results from previous studies have once again been inconsistent; DRB1*0405 and DQB1*0401 are associated with an accelerated progression to cirrhosis while DRB1*0901 and DQB1*0303 appear to protect against the development of cirrhosis in Japanese patients (Aikawa et al 1996). This study did not examine class I alleles. However in several other studies using PCR and sequence specific primers no link between progression of disease and HLA type has been demonstrated (Tibbs et al 1996, Zavaglia et al 1998). Therefore the association of HLA phenotype with progression of disease in chronic HCV infection is perhaps more controversial than the link between phenotype and protection from development of the disease.

The mechanism by which HLA class II molecules affect the outcome after infection with HCV is also unclear. However it is likely that that it is related to the function of HLA molecules in the host immune response. HLA class II recognition is necessary for T helper cell activation. In HCV infection the T cell mediated response is believed to play an important role in liver damage. It may be that differing ability of HLA molecules to bind and present viral antigens results in the differences in immune response such that certain HLA types confer protection from or susceptibility to the development, and progression of this infection.

In conclusion, we have demonstrated that HLA class I and particularly class II antigens may influence the hosts' susceptibility or resistance to HCV. In vitro investigations using HCV antigen presentation to T cells through HLA molecules may ultimately aid in the development of immunotherapy or vaccination in HCV infection.

CHAPTER 4

QUALITY OF LIFE IN PATIENTS WITH CHRONIC HEPATITIS C INFECTION

4.1 Introduction

Quality of Life is a qualitative multidimensional concept that theoretically incorporates a quantitative assessment of an individuals well being. In 1947 the World Health Organisation defined health as 'a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity' (Constitution of World Health Organisation). The term Health related Quality of Life (HRQOL) is now commonly used and numerous generic and disease specific instruments have been developed to measure it. The realisation that objectively measured disease does not necessarily bear a direct relationship to subjectively experienced illness has, in part, led to this rapid expansion of interest. There is, however, concern about the significance of Quality of Life data and it is clear that Quality of Life instruments need to be both valid and reliable (Fallowfield 1996).

Early prospective studies of chronic hepatitis C virus (HCV) infection suggested that it was a benign asymptomatic disease with no significant effects on long-term mortality (Seeff et al 1992). However it is now well established that chronic HCV infection is directly associated with the development of chronic hepatitis, cirrhosis, hepatocellular carcinoma (Fattovich et al; 1997, Alter et al 1992, Hopf et al 1990, Di Bisceglie et al 1991, Takahasi et al 1993, Tong et al 1995) as well as diverse extrahepatic manifestations (Agnello et al 1992, De Castro et al 1993, Johnson et al 1993). Therefore it might be expected that this chronic progressive disease would have a direct impact on Quality of Life.

In this study, we aimed to measure Quality of Life in a Scottish population of HCV infected patients and to compare the results with a healthy control population

4.2 Subjects and methods

4.2.i Subjects

The population studied consisted of three groups:

HCV infected patients

One hundred consecutive patients with chronic HCV infection attending our chronic hepatitis clinic were recruited for the study. All were HCV-Ab and RT-PCR positive for HCV RNA. All patients approached to take part in the study agreed.

No patients were being treated with alpha-interferon at the time of the study.

Four questionnaires were used. The format of them was explained at the clinic and the subjects asked to return them by post. A single postal reminder to those patients who had not returned their questionnaire by 3 weeks after their clinic attendance was sent.

72 (72%) of patients returned their questionnaires completed.

The routes of transmission were recorded and divided into intravenous drug use (IVDU) (n=32), blood transfusion (n=21), sporadic (n=11), sexual transmission (n=5), others/unknown (n=3).

The liver function tests including alanine aminotransferase (ALT) was measured on the day the questionnaire was handed out.

Age at time of infection (if known) and alcohol intake were recorded. Duration of infection (if known) was calculated.

Of the 72 completed questionnaires 57 patients had previously undergone liver biopsy and the disease stage by fibrosis and inflammation was recorded.

Healthy controls

The healthy controls did not have health problems and were not taking any regular medication. They were matched for age and level of education achieved and the same four questionnaires were used.

All patients gave informed consent and ethical approval was obtained for the study.

4.2.ii Methods

measures

All subjects completed four questionnaires. These four questionnaires were selected for use in this study because they have been previously documented to be valid and reliable and the two Quality of Life questionnaires give a general overall objective assessment of well-being.

The Medical Outcomes Study Short Form 36 (SF36)

The World Health Organisation-Quality of Life Questionnaire (WHO-QOL)

The Hospital Anxiety and Depression Scale (HAD)

The Bentall Inventory (measuring fatigue)

SF-36

This is a 36-item instrument, which takes only 5-10 minutes to complete (Ware et al 1992).

It was originally constructed to survey health status in the American Medical Outcomes Study. It was designed for use in clinical practice and research, health policy evaluations and general population surveys. It includes one multi-item scale that assesses eight health concepts. These eight domains that can be divided into two main categories

physical:

1 physical functioning

2 limitations in physical activities due to health problems

3 bodily pain

4 general health perceptions

mental health

5 social functioning

6 limitations in usual role activities due to emotional problems

7 general mental health (psychological distress and well-being)

8 vitality

Each of the eight domains is scored out of twenty. Higher scores indicate better health.

It was designed for either self-administration by persons of 14 years or older or for administration by a trained interviewer in person or by telephone.

There have been many studies examining the validity and reliability of this questionnaire in a variety of populations.

In one postal survey there was considerable evidence for the reliability of the SF-36 and for construct validity in terms of distinguishing between groups with expected health differences. (Brazier et al 1992).

Cross sectional data from the Medical Outcomes study were analysed to test the validity of the SF-36 scales as measures of physical and mental health. Scales shown to primarily measure physical health (physical function and role physical) best distinguished groups differing in severity of chronic medical condition and were the most valid for pure physical health interpretation. Scales shown to primarily measure mental health: (mental health and role limitations-emotional) best distinguished groups differing in the presence and severity of psychiatric disorders and had the most pure mental health interpretation. The social function, vitality and general health scales measured both physical and mental health and therefore had the most complex interpretation. (McHorney et al 1993).

A further study was carried out to assess the reliability, validity and acceptability of the SF-36 in the NHS in the UK. This reported that the SF-36b satisfied rigorous psychometric criteria for validity and internal consistency. Clinical validity was shown by the distinctive profiles generated for four separate medical conditions each of which differed from that in the general population in a predictable way. (Garratt et al 1994).

Finally a study was carried out to determine the criterion validity of the SF-36 in a large community sample in the U.K. Internal consistency of the domains was found to be high. Criterion validity was assessed by comparing scores for the seven dimensions

assessing functional status and well being with the score from the single general health dimension. Significant trends were observed for decreasing SF-36 scores (indicating greater health problems) with worsening self-rated general health. (Jenkinson et al 1994).

This instrument is included in **appendix 2.**

WHO-QOL

This instrument has been developed by the World Health Organisation to assess quality of life across different cultures. The WHO defines quality of life as an individuals' perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns. It is a broad ranging concept affected in a complex way by the person's physical health, psychological state, level of independence, social relationships, personal beliefs and relationship to salient features of the environment." (WHOQOL group 1993, Kuyken et al 1994).

The WHOQOL-100 contains 100 questions over six broad domains of quality of life within which 24 facets are covered.

The six domains include:

1 physical health

2 psychological

3 level of independence

4 social relationships

5 environment

6 spirituality/religion/personal beliefs

Four items are included for each facet, as well as four general items covering subjective overall QOL and health producing a total of 100 items in the assessment. All items are rated on a five point scale. (Table 4.1)

The WHOQOL instrument is said to place primary importance on the perception of the individual. By focusing on individuals own views of their well-being, the

instruments ask not only about the functioning of people with certain diseases/disorders but also how satisfied the patients are with their functioning and with effects of treatment.

The WHOQOL-100 has been tested using psychometric procedures and shows high levels of reliability and validity. In one study the WHOQOL was completed by a sample of adults who represented 16 disease categories. The scores of the scale were found to discriminate well between sick and well people and to concur with reported health status. It concluded that the WHOQOL shows excellent overall internal consistency reliability and can be used with individuals. The level of reliability extended to all domains and patient subgroups. (Skevington 1999).

Field studies with this instrument are still ongoing however it does appear to have cross-cultural validity and to be a reliable and valid measure of QOL.

The instrument is included in **appendix 2.**

DOMAIN	FACETS INCORPORATED WITHIN DOMAIN
Physical health	Energy and fatigue Pain and discomfort Sleep and rest
Psychological	Bodily image and appearance Negative feelings Positive feelings Self-esteem Thinking, learning, memory and concentration
Level of independence	Mobility Activities of daily living Dependence on medicinal substances and medical aids Work capacity
Social relationships	Personal relationships Social support Sexual activity
Environment	Financial resources Freedom, physical safety and security Health and social care: accessibility and quality Home environment Opportunities for acquiring new information and skills Participation in and opportunities for recreation/leisure Physical environment (pollution/noise/traffic/climate) Transport
Spirituality/religion/personal beliefs	Religion/spirituality/personal beliefs

Table 4.1: Facets of WHOQOL questionnaire

HAD

This is a well-validated measure of anxiety and depression (Zigmond et al 1983). It consists of a brief scale of anxiety and one of depression. There are 14 items on two subscales -7 anxiety items and 7 depression items. It was designed to avoid symptoms that may appear in both anxiety and depression and in tests of reliability and validity scores were not affected by the presence of a physical illness. Items such as insomnia, fatigue, anorexia or weight loss, which could be symptoms of a physical disorder, were excluded.

It is a brief self-assessment questionnaire, which is easily understandable and acceptable to patients. It has been demonstrated to be equally effective in hospital, out-patient or community settings.

There are many data on reliability and validity that are summarised in a previous review. (Hermann 1997).

The instrument is included in **appendix 2.**

BENTALL INVENTORY

In 1993 Bentall published a brief nine-item fatigue inventory which has excellent internal consistency and which is now widely used. (Bentall et al 1993)

This instrument is included in **appendix 2.**

4.2.iii Statistics

Statistical differences between groups were assessed using the Mann Whitney U Test or the Kruskal-Wallis 1-way Anova. In view of the multiple comparisons made the level of significance was calculated as $p<0.008$. Results are shown as median with interquartile ranges.

4.3. Results

Subject Response Rate

Seventy two (72%) of the HCV patients (35 male, 37 female) returned their questionnaires completed whilst 33 (100%) healthy controls (7 male, 26 female) completed questionnaires. 70/72 (97.2%) HCV patients completed their questionnaires fully. and 31/33 (94%) of healthy controls completed all parts of the questionnaires.

There was no significant difference between age and level of education achieved between the two groups. Table 4.2.

4.3.i Interpopulation analysis by measure of QOL

(1) SF-36

The subpopulation of patients with HCV infection scored significantly lower (i.e. their health was worse) scores in all eight domains of the SF-36 when compared with healthy controls. (table 4.3).

	Age (mean + SEM)	level of education achieved
Healthy controls	39.6 (2.0)	Primary school 0 (0%) Secondary school 18 (54.5%) University education 15 (45.5%) Postgraduate education 0 (0%)
HCV patients	43.7 (1.5)	Primary school 1 (1.5%) Secondary school 50 (72.5%) University education 17 (24.5%) Postgraduate education 1 (1.5%)

Table 4.2: Demographics of subjects

QOL domain	Median value normal (Interquartile range)	Median value HCV (interquartile range)	P value	Pc
Physical function	100 (10)	87.5 (45)	0.002	*
Role physical	100 (0)	62.5 (100)	<0.001	*
Role emotional	100 (0))	83.35 (100)	<0.001	*
Social function	100 (6.25)	62.5 (50)	<0.001	*
Mental health	80 (14)	72 (36)	0.001	*
Bodily pain	100 (21)	62 (59)	0.001	*
Vitality	80 (17.5)	50 (37.5))	<0.001	*
General health	90 (17.5)	52 (52)	<0.001	*

*=Pc is significant (<0.008)

Table 4.3: SF36 results; HCV versus normal.

(2) WHOQOL

Patients with HCV infection had significantly lower scores (i.e. indicating worse health) in five out of six domains of the WHO-QOL when compared with healthy controls. There was no significant difference between scores for the spirituality facet between the two groups (table 4.5). In addition there was a highly significant difference between the two groups with regard to overall function.

When compared with patients with IDDM the HCV infected patients had significantly lower scores in the categories of physical domain and overall function. There was however no significant difference between the two groups with respect to psychological development, level of independence, social relationships, environment or spirituality. (table 4.6).

QOL domain	Median value **normal** (interquartile range)	Median value **HCV** (interquartile range)	P value	Pc
Physical development	17.0 (2.5)	14.0 (4.6)	<0.001	*
Psychological development	15.6 (3.1)	14.6 (4)	0.023	ns
Level of independence	19.0 (1.5)	15.6 (6.25)	<0.001	*
Social relationships	16.7 (3)	15.7 (3.85)	0.003	*
Environment	16.1 (2.1)	15.0 (3.1)	0.011	ns
Spirituality	14.0 (5)	13.9 (6)	ns	*
Overall function	17.0 (3)	13.5 (6)	<0.001	*

*=Pc is significant ($p<0.008$)

Table 4.4: WHOQOL HCV versus normal

(3) HAD

Although there was a significant difference in the HAD anxiety and depression scores between the HCV infected group and the healthy controls, the median scores for the HCV infected patients, IDDM patients and healthy controls fell within the normal range (less than 7). (table 4.7,4.8).

(4) BENTALL

There was no significant difference in the Bentall scores between the HCV infected and normal population or between the HCV infected patients and IDDM patients. (Figure 4.1).

QOL INSTRUMENT	median score **normal** (interquartile range)	median score **HCV** (interquartile range)	Pc value
HAD A	4 (4.5)	7.0 (7.75)	<0.001
HAD D	1 (3.0)	3.5 (6.5)	<0.001
BENTALL	3 (3.5)	5.0 (10)	ns

Table 4.5:HADS; HCV versus normal

4.3.ii Intrapopulation analysis of patients with chronic HCV infection

Route of transmission of infection

The population of patients with chronic HCV infection were divided according to route of infection. There was no significant difference in QOL scores across all domains of both the SF36 and WHOQOL according to route of infection. (Tables 4.6 and 4.7 respectively). There was also no significant difference in HAD A and D and Bentall scores between different routes of infection.

Alcohol intake

When quality of life and alcohol intake were examined there was no significant difference between alcohol intake and quality of life across all domains and in both the SF36 and WHOQOL questionnaires. (Tables 4.9 and 4.10)

Histology

There was no significant difference between level of fibrosis and quality of life scores across all domains. There was also no significant difference between level of histological inflammation and QOL score. (Table 4.11 and table 4.12)

ALT score

When the HCV infected group were divided according to ALT (normal (<40), moderately elevated (40-80) or high (>80)) there was no significant difference between domain scores and level of ALT.

	IVDU (median and interquartile range)	BLOOD TX (median and interquartile range)	SPORADIC (median and interquartile range)	SEXUAL (median and interquartile range)	Pc value
physical function	92.5 (23.8)	75.0 (65)	95.0 (31.2)	100 (5)	ns
role physical	50.0 (93.8)	25.0 (100)	100 (75)	100 (100)	ns
role emotional	83.5 (100)	83.1 (100)	72.9 (100)	100 (83.5)	ns
social function	81.2 (62.5)	62.5 (50)	81.2 (56.2)	75 (43.8)	ns
mental health	76.0 (38)	74.0 (36)	72.0 (31)	72 (18)	ns
bodily pain	83.0 (58.8)	62.0 (33)	67.0 (66.5)	100 (37.5)	ns
general health	54.7 (53.2)	46.0 (49.5)	62.0 (30)	72 (33.5)	ns
vitality	50.0 (40)	50.0 (33.8)	60.0 (37.5)	70 (35)	ns

Table 4.6: SF36 by route of infection

	IVDU (median and interquartile range)	BLOOD TX (median and interquartile range)	SPORADIC (median and interquartile range)	SEXUAL (median and interquartile range)	Pc value
Physical development	13.5 (4.5)	14.3 (4.2)	15.3 (6)	16.7 (4.2)	ns
Psychological development	15.4 (3.4)	15.5 (5.6)	15.4 (4)	13.8 (2.8)	ns
Level of independence	16.6 (5.9)	15.4 (6.8)	18.2 (5.1)	16.5 (3.4)	ns
Social relationships	16.0 (4.8)	16.0 (6.4)	15.5 (5)	16.0 (2)	ns
Environment	14.8 (2.3)	15.9 (2.2)	15.4 (3.9)	14.5 (2.3)	ns
Spirituality	11.5 (8)	12.5 (6)	14.5 (5.5)	16.0 (8)	ns
Overall function	12.0 (7)	14.0 (4.2)	15.0 (4.5)	14.0 (5)	ns

Table 4.7: WHOQOL by route

	IVDU (median and interquartile range)	BLOOD TX (median and interquartile range)	SPORADIC (median and interquartile range)	SEXUAL (median and interquartile range)	Pc value
HADA	7.0 (8.5)	6 (7.8)	7.5 (8)	5 (5.5)	ns
HADD	3.0 (6.8)	4 (8.2)	2.0 (4.8)	4 (5)	ns
BENTALL	7.5 (10.2)	5 (9.8)	3.0 (9.8)	3 (7.5)	ns

Table 4.8: HAD A and D and BENTALL by route

	minimal (median and interquartile range)	moderate (median and interquartile range)	heavy (median and interquartile range)	Pc value
Physical function	85.0 (40)	90 (67.5)	90.0 (33.8)	ns
Role physical	50.0 (75)	100 (100)	12.5 (100)	ns
Role emotional	100 (83.5)	100 (100)	66.7 (100)	ns
Social function	68.8 (50)	87.5 (50)	62.5 (71.9)	ns
Mental health	72.0 (28)	74 (41)	70.0 (35)	ns
Bodily pain	67.0 (59)	84 (58.2)	51.5 (74)	ns
General health	52.0 (47)	64.5 (65.8)	53.5 (55.8)	ns
Vitality	50.0 (32.5)	60 (55)	50.0 (47.5)	ns

Table 4.9: SF36 by alcohol intake

	minimal (median and interquartile range)	moderate (median and interquartile range)	heavy (median and interquartile range)	Pc value
Physical development	14.0 (3.7)	14.0 (7.2)	14.2 (8)	ns
Psychological development	14.8 (4.4)	15.0 (6.7)	14.5 (3.5)	ns
Level of independence	15.5 (5.2)	16.8 (8.9)	16.0 (8.4)	ns
Social relationships	16.0 (2.9)	15.6 (7.3)	15.2 (2.7)	ns
Environment	15.1 (3.2)	15.2 (4.5)	14.9 (3.2)	ns
Spirituality	13.0 (4.5)	13.5 (9)	11.0 (7.8)	ns
Overall function	14.0 (5)	14.0 (6.5)	12.0 (8)	ns

Table 4.10: WHOQOL by alcohol intake

	Chronic hepatitis (median and interquartile range)	cirrhosis (median and interquartile range)	Pc Value
Physical function	95.0 (27.5)	72.5 (35)	ns
Role physical	100 (75)	0 (93.8)	ns
Role emotional	66.7 (100)	66.5 (100)	ns
Social function	75.0 (50)	56.2 (71.9)	ns
Mental health	72.0 (35)	74.0 (36)	ns
Bodily pain	67.0 (59)	57.5 (53)	ns
General health	62.0 (47)	37.5 (55.8)	ns
Vitality	52.5 (38.8)	50.0 (52.5)	ns

Table 4.11: SF36 by histology

	Chronic hepatitis (median and interquartile range)	cirrhosis (median and interquartile range)	Pc value
Physical development	14.0 (5)	13.7 (7.3)	ns
Psychological development	14.8 (3.6)	15.5 (5.8)	ns
Level of independence	16.4 (5.4)	12.7 (7.7)	ns
Social relationships	15.5 (4.1)	16.2 (5.9)	ns
Environment	15.1 (3)	14.8 (4.6)	ns
Spirituality	13.0 (6.2)	12.0 (6.2)	ns
Overall function	13.0(6)	14.0 (8)	ns

Table **4.12: WHOQOL by histology**

Duration of infection

Patients were also stratified according to duration of infection. (0-10, 11-20, 21-30 and > 30 years). There were no significant differences seen across all domains of both questionnaires.

Age at infection

Age at infection when available was also recorded. Patients were stratified (<25, 26-40 and >40 years old). Those infected over the age of 40 years had significantly worse scores in SF36 physical function and vitality compared to those who were infected under the age of 25 years. (Tables 4.13 and 4.14)

	<25 years (median and interquartile range)	26-40 years (median and interquartile range)	>40 years (median and interquartile range)	Pc Value
Physical function	92.5 (30)	95 (30)	25.0 (52.5)	P=0.001
Role physical	75.0 (100)	100 (100)	25.0 (68.75)	ns
Role emotional	83.4 (100)	100 (33.8)	16.5 (100)	ns
Social function	62.5 (75)	75 (50)	50.0 (31.2)	ns
Mental health	70.0 (36)	76 (32)	64.0 (44)	ns
Bodily pain	68.0 (59)	74 (38)	41.0 (60.5)	ns
General health	47.0 (53.2)	67 (42)	37.0 (31)	ns
Vitality	50.0 (46.2)	55 (35)	32.5 (32.5)	ns

Table 4.13: SF36 by age at infection

	<25 years (median and interquartile range)	26-40 years (median and interquartile range)	>40 years (median and interquartile range)	Pc
Physical development	13.5 (4.8)	14.7 (5.7)	14.0 (2.5)	ns
Psychological development	14.7 (5.2)	15.4 (3.4)	13.6 (5.8)	ns
Level of independence	16.0 (7)	17.5 (4.5)	10.2 (7)	ns
Social relationships	15.3 (5.4)	16.0 (2.3)	14.7 (5.5)	ns
Environment	14.6 (4.2)	15.4 (2.4)	15.6 (2.8)	ns
Spirituality	12.5 (7.5)	13.0 (7)	12.0 (6.5)	ns
Overall function	13.0 (7)	14.0 (6)	13.0 (6)	ns

Table 4.14: WHOQOL by age at infection

4.4 Discussion

In this study we have demonstrated that chronic HCV infection has a statistically significant effect on the majority of domains of Quality of Life as assessed by two separate Quality of Life measures when compared with healthy controls. This difference is not simply explained by anxiety or depression.

There have now been several other studies examining quality of life in patients with HCV infection.

Rodger examined the impact of a diagnosis of HCV infection in an Australian cohort. They used the SF36 questionnaire. They found that patients with a known diagnosis of HCV infection scored worse in 7 out of 8 domains when compared with healthy controls. They also found that patients infected with HCV but unaware of the diagnosis of HCV infection had impaired QOL across 3 of 8 scales compared to healthy controls.(Rodger et al 1999).

Ware et al in 1999 used a different questionnaire the Hepatitis Quality of Life questionnaire part of which was comprised of the SF36. Chronic HCV infected patients had worse scores in physical functioning, role physical, general health, vitality and social functioning when compared to healthy population normals. (Ware et al 1999).

Bonkovsky evaluated 645 patients. They examined QOL before and after treatment with interferon-alpha. They used the SF36 and also incorporated additional items that evaluated perceptions of appetite, cognitive functioning, current health status, feelings of health distress, sexual functioning and sleep quality. Their data indicated that at baseline patients scored low on all measured domains. The greatest reductions were found in those scales that reflected physical impairment. Treatment resulted in

improved scores in those patients who has a sustained biochemical and virological response however those improved scores were still less than those of healthy controls. (Bonkovsky et al 1999).

The sickness impact profile was used by Davis to assess HRQOL in HCV infected patients before and after treatment with alpha-interferon. The authors concluded that patients, before treatment commenced, had significantly worse scores in all categories of the SIP (except eating) when compared with healthy controls. After treatment was completed there was a significant improvement in several domains of the SIP whereas in the untreated control group no significant difference was demonstrated between baseline and the end of treatment values. Further, the authors concluded that although the SIP was a reliable and valid instrument to measure impact of chronic HCV infection on HRQOL, further trials in patients with HCV infection should use a more disease specific and responsive instrument .(Davis et al 1994).

Carithers modified the SF-36 by adding further questions to several of the domains. Psychometric evaluation indicated it to be both reliable and valid. Patients with chronic HCV infection scored significantly lower (i.e. worse) than the general population in each domain and lower than patients with hypertension in 7 of 8 domains. When compared with patients with type II diabetes mellitus they scored significantly lower in the vitality, bodily pain and social function domains, which concurs with our findings. The authors demonstrated no correlation between objective measures of disease activity and severity and subjective findings. (Carithers et al 1996).

The SF36 questionnaire was used in a further study (Foster et al 1998). They found that patients with chronic hepatitis C were polysymptomatic and had significant

reductions in their SF36 scores for all of the modalities tested. This study compared a hepatitis C subpopulation with a hepatitis B subpopulation and demonstrated that although the HBV infected patients had a reduction in the SF36 scores that assessed mental functions, there was no decrease in scores measuring physical symptoms, indicating that the symptoms described by cohorts infected by HBV and HCV are qualitatively different. The authors were also unable to demonstrate a reduction in Quality of Life attributable to the degree of inflammation or the mode of acquisition of the infection.

We used four different instruments to examine Quality of Life as well as anxiety, depression and fatigue. The SF-36 and WHO-QOL measure Quality of Life over a series of independent domains, which together encompass the definition of Quality of Life. This study examined Quality of Life in chronic HCV infection using more than one instrument and our results, which correlate well across both measures used, indicate that Quality of Life is genuinely impaired in patients with chronic HCV infection.

Importantly, the study also demonstrated that there was no significant correlation between route of transmission of infection and QOL score and this implies that it is the HCV infection per se rather than any high risk behaviour (e.g. in IVDU) which affects QOL. This is in agreement with other authors. (Foster et al 1998)

We also examined histological stage of liver disease using degree of fibrosis and inflammation. We found no significant effect on QOL according to either severity of inflammation or fibrosis. There was also no significant effect of presence of cirrhosis on QOL although all the patients with cirrhosis were still well-compensated. This again is in agreement with other investigators, (Foster et al 1998, Carithers et al 1996,

Bonkovsky et al 1999). Duration of infection had no significant effect on scores this would seem logical given that more progressive disease does not appear to be associated with a poorer QOL. However interestingly those infected over the age of 40 years of age had worse scores of physical function and vitality than those infected at a younger age. The reason for this is not entirely clear.

Alcohol intake was also assessed and was found to have no significant effect on QOL scores. This is in concurrence with a recent study in patients with cirrhosis of different aetiologies. (Marchesisi et al 2001).

The observation that Quality of Life scores were not significantly related to ALT is not surprising since there are now many data demonstrating that the ALT titre in chronic HCV infection is not related to disease stage, severity or activity (Puoti et al 1997, Stanley et al 1996).

The third instrument the HAD scale measures anxiety and depression. Although there were significant differences in the scores of the HCV infected group and those of the healthy controls; all the median scores were within the normal range implying that none of the two groups had clinically significant anxiety or depression. This then suggests that the hepatitis C virus per se rather than other factors (e.g. anxiety or depression) impairs quality of Life in affected individuals. Fatigue is a common complaint in many physical diseases and indeed in the general population but is it is particularly common in patients with certain chronic liver disease (e.g. primary biliary cirrhosis). However there was no significant difference demonstrated between the two populations using the Bentall Inventory perhaps because fatigue becomes a more prominent symptom once the liver disease has decompensated. However others more

recently have found a significant difference in fatigue scores using other instruments e.g. the fatigue assessment instrument (Obhrai et al 2001).

What is less clear however, is why there should be such an effect on QOL in these patients. Why does persistent hepatitis C affect how a person feels? It has been postulated that the presence of viraemia and viral replication may be the cause of this impaired QOL. Other theories include release of pro inflammatory cytokines e.g. tumour necrosis factor-α, interleukin1β and interleukin-6 either by the virus itself or by pathophysiological events occurring directly as a result of the infection e.g. hepatocyte damage and necrosis.

In summary we have demonstrated that chronic HCV infection has a significant effect on how patients feel, their ability to function and on their life overall. This effect does not appear to be correlated with disease activity measured biochemically and histologically and is not related to anxiety, depression or fatigue.

Further studies are required to examine the relationship between biological factors (e.g. proinflammatory cytokines), viral factors (e.g. viral load and genotype) and QOL. It is also important to clearly define the effect of treatment on QOL and whether pre-treatment variables have any significant effect on response to treatment.

CHAPTER FIVE

PREVALENCE AND EPIDEMIOLOGICAL CHARACTERISTICS OF HEPATITIS G VIRUS/GB VIRUS C INFECTION IN SCOTTISH BLOOD DONORS

5.1 Introduction

Hepatitis C virus (HCV) has been identified as the principle cause of post transfusion hepatitis (PTH) (Choo et al 1989), while the development of serological tests for blood donor screening (Kuo et al 1989) has led to a dramatic fall in its incidence. However in 10% of cases of PTH and 20% of community-acquired hepatitis no aetiological agent has been identified (Alter et al 1992, Alter et al 1997b). Recently a novel flavivirus, termed GB virus-C was identified using primers derived from conserved regions in the viruses GBV-A and GBV-B which infect tamarinds and other new world primate species (Leary et al 1996). Another group reported the detection and characterisation of hepatitis G virus (HGV) from a patient with non-A, non-B chronic hepatitis who was later found also to be infected with HCV (Linnen et al 1996). GBV-C and HGV have significant nucleotide and amino acid sequence similarities (85%, 97%) and are now accepted to be different isolates of the same virus.

HGV/GBV-C is a positive single-stranded RNA virus with genomic organisation resembling that of the flaviviridae family (Choo et al 1991, Kato et al 1990). However, it appears to differ significantly from HCV in the epidemiology and natural history of infection as well as the consequences of chronic infection. In this study we aimed to determine the prevalence of HGV/GBV-C in large sample of the Scottish donor population, determine the natural history of infection with the virus and assess the clinical significance and possible risk factors for transmission of infection in HGV/GBV-C positive donors.

5.2 Subjects and Methods

5.2.i Subjects.

From October 1996 until December 1996, 1020 blood samples were collected from volunteer blood donors attending the Edinburgh and South East Scotland Blood Transfusion Service centre in Edinburgh. Samples were taken from consecutive regular donors who had given written informed consent (32 of 1052 donors approached did not agree to take part in the study). All subjects were seronegative for human immunodeficiency virus (HIV), hepatitis B virus (HBV) and HCV.

All HGV/GBV-C positive donors were contacted and invited to attend for counselling and further investigation. At their initial consultation repeat sampling for plasma HGV/GBV-C RNA and ALT was undertaken, and a history of risk behaviour sought. In addition a sample of saliva was collected for testing for HGV/GBV-C RNA. Partners of infected donors were also invited to attend. All positive donors were referred to a single hepatologist for clinical evaluation.

Each HGV/GBV-C RNA positive plasma sample was tested for antibodies to HBV core protein (Amerlite anti-HBc assay) and human cytomegalovirus (HCMV, Captia CMV-enzyme immunoassay, Centocor). Approximately one year after initial testing, recalled donors were retested for HGV/GBV-C RNA by PCR.

Archived samples from previous donations were retrieved and tested for HGV/GBV-C RNA. The first two donations were tested (i.e. most distant in time) and if both were negative, intermediate samples were retrieved and tested.

5.2.ii Methods

This work was carried out by Professor Simmonds and his team at the Department of Medical Microbiology, University of Edinburgh.

Detection of HGV/GBV-C RNA and antibody in plasma. Unless otherwise indicated, DNA was extracted directly from 100 ml of EDTA anti-coagulated plasma, and redissolved in 25 ml nuclease-treated water, of which 5 ml was used for PCR. Citrated plasma was collected from blood donors, and combined into pools of 10 before extraction and testing by PCR. Positive pools were subdivided into component donations and re-tested. Initial screening identified 23 positive pools, which led to the identification of one positive component donation in each. RNA was also extracted from 100 ml volumes of saliva samples as described for plasma samples. Viral RNA was amplified by PCR using primers from the 5' non coding region (Jarvis et al 1996). Quantitation was carried out by titration of the cDNA prepared from the nucleic acid as previously described (Jarvis et al 1996). IgG antibodies to GBV-C/HGV E2-antigen were detected using the PLATE Anti-HGenv assay (Boehringer-Mannheim; (Dille et al 1997).

All donors gave informed consent and ethical approval was obtained for the study.

5.3 Results

Prevalence of HGV/GBV-C in blood donors. Twenty-three of 1020 (2.25%) healthy blood donors were positive for HGV/GBV-C RNA in plasma (11 males, mean age-37.9 years and 12 females, mean age-33.3 years). (Table 5.1). Of the 19 who returned for further investigation approximately 10 weeks after donation, 18 remained HGV/GBV-C RNA positive. HGV/GBV-C was detected in 13 of 17 saliva samples from HGV/GBV-C viraemic donors collected at that time, at levels approximately three logs lower than found in plasma (geometric means 7.8×10^3 and 7.4×10^6 copies HGV/GBV-C RNA/ml respectively; Table 1). There was a weak correlation between levels of HGV/GBV-C RNA in plasma and saliva (R=0.442, $p = 0.05$ [Spearman's rank correlation test]). The donor who had become PCR-negative in plasma produced a saliva sample that was PCR-positive (4000 copies HGV/GBV-C RNA/ml).

One year after the original study 11/14 donors who attended for follow-up remained HGV/GBV-C RNA positive. The three donors who became PCR-negative showed the lowest virus loads in plasma at time of original donation (p3: 5×10^5, p18: 5×10^6 and p21: 3×10^4 copies of HGV/GBV-C RNA/ml; $p = 0.017$ [Kruskall-Wallis test]).

Clinical assessment of HGV/GBV-C infected blood donors. Two donors (p13, p20) of the 18 donors recalled for clinical assessment had a previous history of blood transfusion. Several donors had undergone ear piercing or tattoos but in establishments where virus transmission would not be considered likely. None had a

history of intravenous drug use or sexual contact with an individual at high risk of parenteral virus infection.

Fourteen of 23 HGV/GBV-C positive donors were clinically examined. None had any history of jaundice, symptoms or stigmata of chronic liver disease or hepatomegaly. Five of 18 donors reported various musculoskeletal symptoms, although not of a consistent pattern and of uncertain relationship to HGV/GBV-C infection. Liver function tests (LFTs) were carried out for the 19 donors returning after original donation (Table 5.1). Two donors showed a minimal elevation of alanine aminotransferase (ALT; 47 and 43 IU/ml [normal range 10-40 IU/ml]). The median ALT level amongst HGV/GBV-C infected donors was 20 IU/Ml, lower than the median of 32 for a control group of 100 HGV/GBV-C uninfected blood donors (p = 0.015) and a median of 61 for 91 HCV-infected donors ($p < 0.0001$) (McOmish et al 1993). All other LFT tests (bilirubin, alkaline phosphatase and gamma-glutamyl transferase) were similarly within the normal range. All measurements in a full blood count were in the normal range for the 19 donors, apart from mild lymphopoenia in five.

Two partners donated at the time of the original study and tested negative. Three partners attended the counselling and follow-up session, and were negative for HGV/GBV-C RNA in plasma and saliva, and negative for antibody to HGV/GBV-C.

Incidence of HGV/GBV-C infection in blood donors. Archived samples from previous blood donations of all 23 HGV/GBV-C infected donors were tested by PCR (Figure. 5.1). All samples from 6 donors (collected from 1986-1994) were PCR-positive, while the earliest samples from the remaining 17 (1983-1995) were PCR-

negative. Two donors (p10 and p19) became PCR-positive in 1994 and 1992 respectively, but over the next 1-2 years became transiently PCR-negative and negative for antibody to E2, before becoming viraemic again at the end of 1996.

EPIDEMIOLOGICAL AND CLINICAL CHARACTERISTICS OF HGV/GBV-C INFECTED BLOOD DONORS

Donor*	Age	Sex	ALT IU/ml	Previous donations	Plasma copies/ml†	Saliva copies/ml†	Duration infection‡
p12	20	M	25	6	5×10^6	<100	1 year
p11	21	F	18	10	5×10^8	5×10^4	>2 years
p15	25	F	18	12	5×10^7	5×10^3	1 year
p16	26	F	20	17	5×10^8	5×10^4	2 years
p8	27	F	13	6	5×10^7	5×10^4	6 years
p17	27	M		14			>8 years
p6	28	F	18	19	< 100	4×10^3	5 years
p9	28	F	15	9	5×10^6	5×10^4	6 years
p5	29	M	38	6	5×10^7	5×10^5	10 years
p3	30	M	47	24	5×10^5	4×10^3	2 years
p2	32	F		7			9 years
p14	32	M	33	7			>5 years
p4	36	F		6			>11 years
p18	39	M	30	28	5×10^6	5×10^3	6 years
p19	39	F	27	19	5×10^8	5×10^3	8 years
p13	40	F	15	12	5×10^7		4 years
p22	44	M		42			7 years
p7	45	F	19	11	5×10^7	5×10^3	>10 years
p1	47	M	20	24	5×10^6	5×10^5	7 years
p20	48	M	34	47	5×10^7	5×10^3	9 years
p10	50	M	8	8	5×10^7	5×10^4	3 years
p21	51	M	43	56	3×10^4	<100	9 years
p23	53	F	31	47	5×10^6	5×10^5	9 years

* Donors ranked by age

† HGV/GBV-C virus load in plasma and saliva from donor upon recall

‡ Approximate duration of HGV/GBV-C infection; time of infection calculated as mid-point between last PCR negative and first PCR positive donation; in most cases these are minimum estimates as donors have been followed prospectively for at longest 1 year (Fig.5.1).

Table 5.1

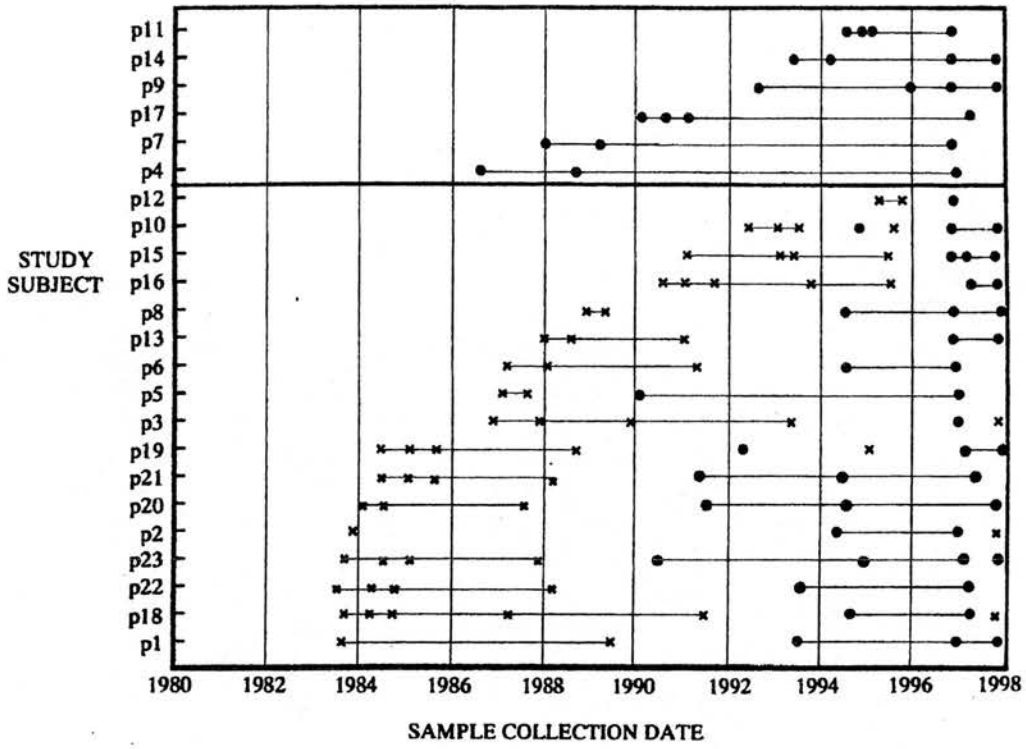


Figure 5.1. Retrospective testing for HGV/GBV-C-RNA of archived blood donations from 23 blood donors identified as HGV/GBV-C-infected in November-December 1996. 6 donors above horizontal line are those with persistent infection throughout the observation period. •, HGV/GBV-C PCR-positive; ×, PCR-negative.

A total of 17 blood donors became infected with HGV/GBV-C over a mean observation period of 9.7 years. The number of susceptible blood donors investigated was 1046 (excluding the persistently viraemic donors during the observation period). Therefore, an approximate calculation of the incidence of HGV/GBV-C infection would be 0.17% per year.

A technical explanation for the large number of HGV/GBV-C negative samples collected before 1990 is that HGV/GBV-C RNA sequences had degraded upon long-term storage, although positive samples from individuals (p4 and p7) were recorded before this date. Although this hypothesis was not experimentally tested, it was possible to re-calculate the incidence of HGV/GBV-C infection using shorter time intervals. Using only the results from 1990 onwards, and assuming that HGV/GBV-C infection occurred at the mid-point between the last PCR negative and first PCR positive sample, a total of 13 blood donors became infected over a mean interval of 6.2 years, producing an annualised incidence of 0.20% per year). Similarly, 8 became positive from 1992 onwards (mean observation period 4.5 years, incidence 0.17% per year). The similarity in these estimates suggests that degradation of HGV/GBV-C RNA storage did not affect the calculation of incidence.

A final qualification in interpreting the incidence calculation arises from the assumption that all donors (other than those who were persistently infected) are susceptible to HGV/GBV-C infection. The proportion of donors with past resolved infection with HGV/GBV-C (and who may be anti-E2 positive) was not determined in this study, although other studies of similar donor populations have documented frequencies of 7.4%-16% (Lou et al 1997, Tacke et al 1997, Feucht et al 1997). The estimates for incidence presented above apply to the blood donor population as a

whole, and would therefore be higher in the subset of individuals without prior exposure to HGV/GBV-C.

5.4 Discussion

Prevalence and incidence of HGV/GBV-C infection. In this study we have demonstrated that 23/1020 (2.25%) of healthy non-remunerated blood donors had plasma samples positive for HGV/GBV-C RNA by RT PCR. This frequency lies within the range reported for comparable non-remunerated blood donor populations in other countries such as Germany (1.3%-1.9%, (Feucht et al 1997, Rith et al 1997)), France (4.2%, (Loiseau et al 1997), USA (1.7%, (Linnen et al 1996)), Australia (4%, (Moaven et al 1996)) and Japan (0.5%-1.2%, (Yoshikawa et al 1997, Orita et al 1996)). The male-female ratio of HGV/GBV-C infected donors was 11:12, different from the 3:1 ratio observed in amongst blood donors infected with HCV in Scotland (McOmish et al 1993) or elsewhere. The high prevalence of HGV/GBV-C infection, the different male/female ratio, and the absence of significant disclosed parenteral risk factor for infection in the HGV/GBV-C infected donors strongly suggests other routes of transmission.

Amongst other proposed routes of transmission of HGV/GBV-C, evidence for transmission by sexual contact includes the finding of high prevalence of infection in individuals with sexually transmitted disease, or with other evidence of sexual exposure with multiple partners (Stark et al 1996, Kao et al 1997, Wu et al 1997, Scallan et al 1998). Against this hypothesis is the absence of detectable transmission of HGV/GBV-C (either by PCR or serology) to the sexual partners of 5 infected donors. Mother to child transmission has been documented by the frequent detection of HGV/GBV-C viraemia (20-70%) in children born from infected mothers (Moaven et al 1996, Viazov et al 1997, Feucht et al 1996, Fischler et al 1997), and the genetic

identity of HGV/GBV-C maternal and infant strains (Viazov et al 1997). However, the observation of *de novo* infection in at least 17 of the 23 HGV/GBV-C infected blood donors in the current study indicates the acquisition of infection occurred predominantly in adulthood.

The frequent finding of HGV/GBV-C positive samples amongst archived donations from the infected donors indicates that infection may persist for several years (at least 10 years in p4). The results of this retrospective study are consistent with the finding of persistent infection of at least 2 years amongst 4 of 5 Australian blood donors (Moaven et al 1996), and the documented prolonged infection in varying proportions of individuals infected with HGV/GBV-C through blood transfusion or treatment with blood products (Roth et al 1997, Hwang et al 1997, Lefrere et al 1997, Hanley et al 1998).

A surprising finding that arose from the retrospective study was the relatively high incidence of HGV/GBV-C infection. The calculated rate (170-200 infections per 100,000 person years) contrasts strongly with rates of acquisition of HCV (1.8-10 per 100,000, (Prati et al 1997, Riggert et al 1996, Saski et al 1996, Itoh et al 1996, Whyte et al 1997)), HIV (1.3 - 4.0 per 100,000, (Itoh et al 1996, Whyte et al 1997, Remis et al 1997)) and HBV (1.7 - 4.0, (Itoh et al 1996, Whyte et al 1997)) in similar non-remunerated blood donor populations in Europe, USA, Japan and Australia. If in the future, donations were to be screened for HGV/GBV-C RNA by PCR, the number of new infections detected would place severe constraints on the size of the pool used. For example, even if all persistently infected donors were excluded, new infections with HGV/GBV-C over a mean donation interval of 6 months would lead to

contamination of 50% of pools containing 500 component donations, and 10% of pools containing 100 components.

Clinical assessment of HGV/GBV-C infected donors. The asymptomatic nature of HGV/GBV-C was directly demonstrated by our clinical examination of the infected donors. Two donors showed minimal elevation of ALT levels, but the mean level (20 IU/ml) and all other liver function tests were in the normal range. No subject had a previous history of acute hepatitis or jaundice. No donor had any abnormality on clinical examination, other than non-specific musculoskeletal symptoms in 5 individuals. However there was no consistent pattern of joint involvement, and larger studies would be required to make a more substantive link.

These findings are consistent with the absence of clinically or biochemically apparent liver disease in those infected by blood transfusion (Alter et al 1997b, Wang et al 1996, Yashina et al 1997). For example, of 79 cases of PTH, only three patients were infected with HGV/GBV-C alone (Alter et al 1997b), all had mild, asymptomatic hepatitis, and the absence of any correlation between ALT elevation and detection of HGV/GBV-C by PCR suggests other causes for the observed hepatitis. In another study only 1/48 patients with acute non A-E hepatitis had detectable HGV/GBV-C RNA levels by RT PCR (Nakatsuji et al 1996).

Although early reports indicated a significantly higher frequency of GBV-C/HGV infection in patients with non A-non E hepatitis (Linnen et al 1996, Fioradalisi et al 1996, Colombatto et al 1996), later studies have largely discounted this association (Feucht et al 1997, Moaven et al 1996, Matsumura et al 1997, Alter et al 1997a, Sarrazin et al 1997, Sugai et al 1997, Tanaka et al 1997, Thomas et al 1997, Zhang et

al 1997, Ross et al 1997). In patients with dual infection with HGV/GBV-C and HCV, HGV/GBV-C had no effect on clinical outcome and ALT levels correlated with HCV rather than HGV/GBV-C levels. In the 16/519 HGV/GBV-C positive haemodialysis patients none had raised ALT levels despite having documented persistent infection for up to 16 years (Masuko et al 1996). Viruses that are able to establish persistent infection often produce chronic disease, but there are no prospective studies that document progression of HGV/GBV-C to cirrhosis, hepatocellular carcinoma or decompensated liver disease, in marked contrast with chronic HCV infection (Seeff et al 1992).

A difficulty encountered in investigating the HGV/GBV-C infected donors in the current study arose from the current lack of information about the tropism and likely disease associations of GBV-C/HGV *in vivo*. Although HGV/GBV-C RNA can be detected in the liver (Saito et al 1997), there is little evidence for its replication there (Laskus et al 1997, Kudo et al 1997, Mellor et al 1998). Thirteen of seventeen of our HGV/GBV-C positive donors showed relatively high levels of HGV/GBV-C RNA in saliva, and a mean ratio to levels detected in plasma of approximately 1:1000. The frequency of detection and ratio to levels detected in plasma contrasts with that documented for HCV, and suggests that replication of HGV/GBV-C in the respiratory or gastrointestinal tracts may occur. None of them had any history of respiratory symptoms or disease, although its secretion into saliva may represent a route of transmission, as documented for HCMV and other herpes viruses.

In conclusion this study demonstrates that HGV/GBV-C infection is common amongst our donor population but is not associated with significant hepatic disease or symptoms. We have not been able to identify a risk factor for infection in the majority

of HGV-positive donors. HGV/GBV-C RNA was present in the plasma in the majority of subjects for several years and was likely to have been transfused in a large number of blood components. These findings will enable recipients of HGV/GBV-C positive blood to be identified and the clinical sequelae investigated. In the interim, in the absence of clinicopathological data suggesting significant morbidity in HGV/GBV-C infected donors, blood donors should not be screened for HGV/GBV-C.

CHAPTER 6

DISCUSSION AND SUGGESTIONS FOR FUTURE WORK

Hepatitis C virus has dominated the practice of clinical hepatology since its discovery in 1989. It is common, predominantly transmitted parenterally and can be silent for many years only becoming apparent when clinically significant complications occur. It is the most common cause of post transfusion hepatitis worldwide and is now a leading cause of liver disease requiring OLT.

Current treatment strategies are still not 100% effective and an effective vaccine is not imminently available. Disease progression is not uniform. Establishing those factors associated with accelerated progression or a more benign course of disease is therefore of clinical relevance.

This thesis examined some of the factors important in progression of disease in this cohort of patients. In **chapters 2 and 3** various factors were examined. In keeping with the majority of regions in the United Kingdom the most common **route of transmission** of infection was through intravenous drug use. This is reflected in the low mean age at infection. In this cohort route of infection did not appear to play a significant role in progression to cirrhosis. There are conflicting results with some studies demonstrating that infection through blood transfusion is associated with more severe histological findings. (Gordon et al 1997). More recent work has concluded that route of infection does not influence progression to cirrhosis.

In interpreting these results it must be recognised that the patients studied may not reflect the general HCV population. This may occur as a result of particular referral patterns to a tertiary referral centre. In **chapter 4** route of infection did not have any significant association with any domain of QOL. This was unexpected given the perceived chaotic lifestyle and other comorbidity associated with intravenous drug

use. Selection bias may account for this finding. Patients attending clinics may be better motivated and are less likely to be currently abusing drugs.

Alcohol consumption was strongly associated with increased fibrosis and progression to cirrhosis. This has been previously reported by many authors (Poynard et al 1997, Wiley et al 1998) and discussed in more detail in **chapter 2**. When QOL was measured in **chapter 4** alcohol intake had no significant effect on any of the QOL domains. This implies therefore that although excess alcohol consumption is associated with a more rapid progression of disease it is not associated with a diminished QOL.

ALT values in **chapter 2** had no apparent effect on inflammation or fibrosis. It has now been conclusively shown that ALT levels are a poor indicator of hepatic histology. (Healey et al 1995, Naito et al 1994, Bruno et al 1994). However ALT values have been shown to be associated with progression of disease. Raised ALT has now been reported to be associated with a more rapidly progressive disease (Ghany et al 2000, Marcellin 2002) and normal ALT is associated with a slower rate of progression to cirrhosis (Freeman et al 2001). Because the biopsies in the cohort in chapter 2 were not done sequentially, rate of progression to cirrhosis related to level of ALT cannot be assessed in this population.

When HLA status was analysed in **chapter 3** HLA DR6 was associated with persistently normal transaminases and HLA B7 and DR7 with abnormal transaminases. This may therefore suggest that DR6 is associated with a slower rate of progression to cirrhosis and B7 and DR7 are associated with a more rapid progression to cirrhosis given the studies reported above. It would require histological

confirmation to be absolutely certain. When ALT values were examined in relation to QOL in **chapter 4** no significant relationship was found.

Age at time of infection was also shown in **chapter 2** to be associated with a more rapid progression to cirrhosis. The reasons for this are not entirely clear but may be related to impaired immunity in older people. When QOL was examined in chapter 4 those aged over 40 years old at time of infection had worse scores in physical function and vitality compared to younger patients. What needs to be ascertained is the reason for accelerated progression in older people as this may help to explain the impaired QOL in these patients.

Male sex was also associated with an increased degree of liver fibrosis. Other authors have reported similar findings. (Poynard et al 1997).

In **chapter 3** the role of HLA status in progression to cirrhosis was examined. HLA B35 was found to protect against the development of cirrhosis. HLA is a critical genetic factor that determines individual variations of the immune response to antigens. As previously discussed in **chapter 1** The T cell mediated response is important in the development of hepatocellular damage and persistence of infection and it might be speculated that HLA type affects the immune response in certain patients with HCV infection thus resulting in more rapidly progressive disease.

One of the major findings in this study was that HLA DR5 appeared to protect against the development of chronic disease as none of our HCV infected patients in the HLA study had the DR5 allele.This concurs with other studies. (Zavaglia et al 1996, Thursz et al 1996). A further study found that the presence of DR5 was associated with a benign course of liver disease in Caucasians. (Peano et al 1994). The mechanism by which the HLA class II molecules confer protection against or susceptibility to

chronic hepatitis C is probably related to the function of these molecules in the immune responses and their differing ability to bind and present viral peptides. However the evidence for importance of HLA antigens in viral hepatitis C is conflicting and various other studies have found relationships between other HLA types and protection from and susceptibility to progressive disease. (Czaja et al 1995, Zavaglia et al 1998, Kuzushita et al 1996, Verdon et al 1994, Vitte et al 1995). Serotyping has now largely been superceded by DNA typing which is known to be superior e.g. genomic typing of the gene coding for HLA DR11 at serological level may yield up to 16 alleles in different ethnic groups. (Zavaglia et al 1998). Ideally DNA typing would have been used in this study for the reasons discissed in **chapter 3.** A further source of potential bias is the relatively small number of patients studied and it would be worthwhile repeating the study using DNA typing and a significantly larger study group.

In **chapter 4** quality of life in HCV was examined. There are many studies now documenting the progressive nature of chronic HCV infection. However there have been fewer studies examining the impact of this chronic disease on patients well being and QOL. Part of the problem is developing appropriate instruments to measure any impairment present and determining whether the instrument used are reliable and valid and therefore measure what you are aiming to measure. This study clearly demonstrated, using several reliable and well-validated instruments, that QOL is impaired across several physical and mental domains in patients with chronic HCV infection. The exact reasons for this are less clear. It has been shown in several studies that QOL improves after treatment. Ware et al 1999, Bonkovosky et al 1999).

In addition the diagnosis of inflammation and/or fibrosis on liver biopsy has previously been used as an indication of who to treat. In **chapter 4** it was demonstrated that the presence of inflammation on biopsy does not correlate with QOL. Part of the aim of treatment is obviously to improve symptoms and this implies that the use of objective markers alone not sufficient. Also consideration must be given to the possibilty of treatment for patients who may not have histologically progressive or severe disease but who have debilitating symptoms and impaired QOL. Should people be treated on the basis of symptoms and QOL rather than on histological, virological and biochemical markers?

In **chapter 5** a novel flavivirus HGV was investigated. When HCV was identified it became apparent that it did not account for all the cases of PTH and community acquired non A, non-B hepatitis. The remaining group has been termed non A-E hepatitis and is believed to have a viral aetiology. Linnen et al cloned HGV from the serum of a patients with non-A non-B hepatitis (Linnen et al 1996) whilst Leary et al identified the isolate GBV-C. They isolated three viral agents from the serum of a tamarin inoculated with serum from a surgeon who had contracted hepatitis. GBV-C was thought to be the likely viral hepatitis candidate in humans. (Leary et al 1996). Sequence comparisons of the two viruses revealed that they were isolates of the same viruses and had similarities to other members if the flaviviridae such as HCV. Was this virus then responsible for the undiagnosed cases of non A-E hepatitis? Early studies suggested that HGV RNA was present in 14% of patients with non A-E hepatitis however similar numbers of patients with acute hepatitis A infection also had HGV-RNA in their serum. (Karayiannis et al 1997b). Prospective studies have shown that HGV can be transmitted by blood transfusion. Other studies have documented high

prevalence of HGV-RNA in people with frequent parenteral exposure including IVDU, haemodialysis patients and patients with haemophilia. (Hadziyannis et al 1995, Aikawa et al 1996, Masuko et al 1996, Jarvis et al 1996). Mother to infant and sexual transmission have also been demonstrated. (Feucht 1996, Scallan et al 1998). The role of HGV in acute fulminant hepatitis is more controversial with initial reports suggesting a direct link (Yoshiba et al 1995) however subsequent studies would suggest there is no significant role for HGV in fulminant hepatitis. (Haydon et al 1997, Sallie et al 1996, Kuroki et al 1996). On this background the study in **chapter 5** aimed to determine the prevalence of HGV in a population of blood donors to determine the natural history of infection and assess its clinical significance. A prevalence rate of 2.25% was found. This is in keeping with other studies of blood donor prevalence in Northern Europe. HGV-RNA was found to be persistent in plasma over time and HGV-RNA was found in the saliva in many of the donors positive for HGV-RNA. The majority of donors had no clinical or biochemical evidence of liver disease. There was no evidence of sexual transmission in this small cohort of infected donors. If all donors with HGV-RNA were to be excluded from donating this would result in a significant reduction in the number of donated blood units able to be used. In this climate of falling blood donations this would potentially have serious implications. On balance with all the evidence available pointing to the fact that HGV does not appear to cause significant liver disease there seems little justification in routinely testing all blood donors for HGV-RNA.

It is still important to determine whether HGV is a hepatotropic virus or whether it is a virus with other tissue tropism. Does HGV cause any clinically significant disease? It is also important to develop an appropriate serological test to identify those with

previous infection and without current viraemia as this would aid in studies of the long-term implications of this seemingly innocuous virus. It does however imply that there is another virus, not yet discovered, that is the cause for the remaining cases of non-A-E hepatitis.

Suggestions for future work

There are now more successful treatment modalities available for patients with chronic HCV infection. Novel ways of successfully treating those who do not respond to standard combination therapy with ribavirin and interferon-alpha are required. Further studies examining different dose and duration regimens are necessary as well as examining the efficacy of other agents either as combination therapy or as part of a triple therapy regime.

In HLA studies the gold standard test is DNA typing. We need to perform larger studies using this technique in our population and also assessing HLA status with more accurate markers of liver inflammation and fibrosis.

In Quality of Life there is much to be learned. The mechanisms of impaired QOL need to be determined as they are poorly understood. More studies need to be carried out examining the effect of treatment on QOL and to assess whether the improvement, which has been documented in the initial studies done, is maintained over significant periods of time e.g. 5 years.

Finally with HGV many of the studies have now been completed. It is important to further investigate the effects of the HGV elsewhere on the body and any long-term effects as this would potentially have significant implications for blood donor

screening. The search for the new virus for non-A-E continues and although other viruses have been postulated, e.g. TTV, the culprit has not yet been identified.

CHAPTER 7

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APPENDIX 1

HEPATITIS C DATABASE

NAME

UNIT NUMBER

ADDRESS

TEL NO

SOURCE OF REFERRAL

MODE OF INFECTION

YEAR OF INFECTION

HCV-AB DATE

RIBA 3 DATE

BANDS

LIVER BIOPSY DATE
BX NO MACROSCOPIC

HISTOLOGICAL
HEPATITIS-
- PORTAL INFLAMMATION
- LOBULAR INFLAMMATION
- FIBROSIS
- FAT

CIRRHOSIS

GENOTYPE

ALCOHOL INTAKE

OTHER VIRAL INFECTIONS

HBV	e ag	s ag	antiHBc	antiHBe	antiHBs
HDV					
HIV					

OTHER MEDICAL NOTES

hepatotoxic drugs
autoantibodies

INTERFERON TREATMENT

DATES OF RX

DOSE GIVEN

REASON RX STOPPED

SIDE EFFECTS

REASON NOT GIVEN TREATMENT

ALT

DATE						
LEVEL						

DATE						
LEVEL						

HCV-RNA

DATE						
RESULT						
CL BL NO						

LFTS

DATE							
GGT							
ALKP							
BIL							
ALB							

FBC

DATE							
WCC							
HB							
PLAT							
MCV							

FERRITIN

DATE							

SYMPTOMS AT DIAGNOSIS

SIGNS AT DIAGNOSIS

1) DECOMPENSATED LIVER DISEASE

2) JAUNDICE

3) SIGNS OF CHRONIC LIVER DISEASE (LIST)

4) HEPATOMEGALY
   SIZE
   DESCRIPTION

5) SPLENOMEGALY

6) OTHER RELEVANT SIGNS

PREVIOUS HISTORY OF ACUTE HEPATITIC EPISODE

ULTRASOUND SCAN RESULT

APPENDIX 2

SF - 36 HEALTH SURVEY DATE..... ID / RNO.....

Instructions : This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities.

Answer every question by marking the answer as indicated. If you are unsure about how to answer a question, please give the best answer you can.

1 In general, would you say your health is :-

(circle one)

Excellent 1
Very Good 2
Good 3
Fair 4
Poor 5

2 Compared to one year ago how would you rate your health now ?

(circle one)

Much better now than one year ago 1
Somewhat better now than one year ago 2
About the same as one year ago 3
Somewhat worse now than one year ago 4
Much worse now than one year ago 5

3 The following questions are about activities you might do during a typical day. Does your health now limit you in these activities. If so, how much ?

(circle one number on each line)

ACTIVITIES	Yes, Limited A Lot	Yes, Limited a Little	No, Not Limited At All
Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	1	2	3
Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling or playing golf	1	2	3
Lifting or carrying groceries	1	2	3
Climbing several flights of stairs	1	2	3
Climbing one flight of stairs	1	2	3
Bending, Kneeling or Stooping	1	2	3
Walking more than one mile	1	2	3
Walking half a mile	1	2	3
Walking one hundred yards	1	2	3
Bathing or dressing yourself	1	2	3

4 During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health ?

(circle one number on each line)

	YES	NO
Cut down on the amount of time you spent on work or other activities	1	2
Accomplished less than you would like	1	2
Were limited in the kind of work or other activities	1	2
Had difficulty performing the work or other activities (for example, it took extra effort)	1	2

5 During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious) ?

(circle one number on each line)

	YES	NO
Cut down on the amount of time you spent on work or other activities	1	2
Accomplished less than you would like	1	2
Didn't do work or other activities as carefully as usual	1	2

6 During the past 4 weeks, to what extent has your physial health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups ?

(circle one)

Not at all 1
Slightly 2
Moderately 3
Quite a bit 4
Extremely 5

7 How much bodily pain have you had during the past 4 weeks ?

(circle one)

None 1
Very mild 2
Mild 3
Moderate 4
Severe 5
Very severe 6

8 During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework) ?

(circle one)

Not at all 1
A little bit 2
Moderately 3
Quite a bit 4
Extremely 5

9 These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling.
How much of the time during the past 4 weeks.

(circle one number on each line)

	All of the Time	Most of the Time	A good bit of the Time	Some of the Time	A little of the Time	None of the Time
Did you feel full of life ?	1	2	3	4	5	6
Have you been a very nervous person ?	1	2	3	4	5	6
Have you felt so down in the dumps that nothing could cheer you up ?	1	2	3	4	5	6
have you felt calm and peaceful ?	1	2	3	4	5	6
Did you have a lot of energy ?	1	2	3	4	5	6
Have you felt downhearted and low ?	1	2	3	4	5	6
Did you feel worn out ?	1	2	3	4	5	6
Have you been a happy person	1	2	3	4	5	6
Did you feel tired ?	1	2	3	4	5	6

10 During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc) ?

(circle one)

All of the time 1
Most of the time 2
Some of the time 3
A little of the time 4
None of the time 5

11 How TRUE or FALSE is each of the following statements for you ?

(circle one number on each line)

	Definitely True	Mostly True	Don't Know	Mostly False	Definitely False
I seem to get ill more easily than other people	1	2	3	4	5
I am as healthy as anybody I know	1	2	3	4	5
I expect my health to get worse	1	2	3	4	5
My health is excellent	1	2	3	4	5

Field Trial

WHOQOL-100

February 1995

Instructions

This questionnaire asks how you feel about your quality of life, health, and other areas of your life. Please answer all the questions. If you are unsure about which response to give to a question, please choose the one that appears most appropriate. This can often be your first response.

Please keep in mind your standards, hopes, pleasures and concerns. We ask that you think about your life in the **last two weeks.**

For example, thinking about the last two weeks, a question might ask:

How much do you worry about your health?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

You should circle the number that best fits how much you have worried about your health over the last two weeks. So you would circle the number 4 if you worried about your health "Very much", or circle number 1 if you have worried "Not at all" about your health. Please read each question, assess your feelings, and circle the number on the scale for each question that gives the best answer for you.

Thank you for your help

The following questions ask about **how much** you have experienced certain things in the last two weeks, for example, positive feelings such as happiness or contentment. If you have experienced these things an extreme amount circle the number next to "An extreme amount". If you have not experienced these things at all, circle the number next to "Not at all". You should circle one of the numbers in between if you wish to indicate your answer lies somewhere between "Not at all" and "Extremely". Questions refer to the last two weeks.

F1.2 (F1.2.1)* Do you worry about your pain or discomfort?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

F1.3 (F1.2.3) How difficult is it for you to handle any pain or discomfort?

Not at all	Slightly	Moderately	Very	Extremely
1	2	3	4	5

F1.4 (F1.2.5) To what extent do you feel that (physical) pain prevents you from doing what you need to do?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

F2.2(F2.1.3) How easily do you get tired?

Not at all	Slightly	Moderately	Very	Extremely
1	2	3	4	5

F2.4 (F2.2.4) How much are you bothered by fatigue?

Not at all	Slightly	Moderately	Very	Extremely
1	2	3	4	5

F3.2 (F4.1.3) Do you have any difficulties with sleeping?

None at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

F3.4 (F4.2.3) How much do any sleep problems worry you?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

F4.1 (F6.1.2) How much do you enjoy life?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

* The numbers in brackets refer to the number of the question in the pilot question pool. National versions must be constructed using that same question taken from national version of the pilot questionnaire.

F4.3 (F6.1.4) How positive do you feel about the future?

Not at all	Slightly	Moderately	Very	Extremely
1	2	3	4	5

F4.4 (F6.1.6) How much do you experience positive feelings in your life?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

F5.3 (F7.1.6) How well are you able to concentrate?

Not at all	Slightly	Moderately	Very well	Extremely
1	2	3	4	5

F6.1 (F8.1.1) How much do you value yourself?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

F6.2 (F8.1.3) How much confidence do you have in yourself?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

F7.2 (F9.1.3) Do you feel inhibited by your looks?

Not at all	Slightly	Moderately	Very much	Extremely
1	2	3	4	5

F7.3 (F9.1.4) Is there any part of your appearance which makes you feel uncomfortable?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

F8.2 (F10.1.3) How worried do you feel?

Not at all	Slightly	Moderately	Very	Extremely
1	2	3	4	5

F8.3 (F10.2.2) How much do any feelings of sadness or depression interfere with your everyday functioning?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

F8.4 (F10.2.3) How much do any feelings of depression bother you?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

F10.2 (F12.1.3)To what extent do you have difficulty in performing your routine activities?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

F10.4 (F12.2.4)How much are you bothered by any limitations in performing everyday living activities?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

F11.2 (F13.1.3)How much do you need any medication to function in your daily life?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

F11.3 (F13.1.4)How much do you need any medical treatment to function in your daily life?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

F11.4(F13.2.2) To what extent does your quality of life depend on the use of medical substances or medical aids?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

F13.1(F17.1.3)How alone do you feel in your life?

Not at all	Slightly	Moderately	Very much	Extremely
1	2	3	4	5

F15.2 (F3.1.2) How well are your sexual needs fulfilled?

Not at all	Slightly	Moderately	Very much	Extremely
1	2	3	4	5

F15.4 (F3.2.3) Are you bothered by any difficulties in your sex life?

Not at all	Slightly	Moderately	Very	Extremely
1	2	3	4	5

F16.1(F20.1.2)How safe do you feel in your daily life?

Not at all	Slightly	Moderately	Very	Extremely
1	2	3	4	5

F16.2(F20.1.3)Do you feel you are living in a safe and secure environment?

Not at all	Slightly	Moderately	Very much	Extremely
1	2	3	4	5

F16.3(F20.2.2)How much do you worry about your safety and security?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

F17.1(F21.1.1)How comfortable is the place where you live?

Not at all	Slightly	Moderately	Very	Extremely
1	2	3	4	5

17.4(F21.2.4)How much do you like it where you live?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

18.2(F23.1.5)Do you have financial difficulties?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

18.4(F23.2.4)How much do you worry about money?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

19.1(F24.1.1)How easily are you able to get good medical care?

Not at all	Slightly	Moderately	Very	Extremely
1	2	3	4	5

21.3(F26.2.2)How much do you enjoy your free time?

Not at all	A little	Moderately	Very much	An extreme amount
1	2	3	4	5

22.1(F27.1.2)How healthy is your physical environment?

Not at all	Slightly	Moderately	Very	Extremely
1	2	3	4	5

22.2(F27.2.4)How concerned are you with the noise in the area you live in?

Not at all	A little	Moderately	Very much	An extreme amount
1	2	3	4	5

23.2(F28.1.4)To what extent do you have problems with transport?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

23.4(F28.2.3)How much do difficulties with transport restrict your life?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

Note: These questions were inappropriately given a capacity response scale in the pilot version. They are to be given an intensity scale in the WHOQOL-100.

The following questions ask about **how completely** you experience or were able to do certain things in the last two weeks, for example activities of daily living such as washing, dressing or eating. If you have been able to do these things completely, circle the number next to "Completely". If you have not been able to do these things at all, circle the number next to "Not at all". You should circle one of the numbers in between if you wish to indicate your answer lies somewhere between "Not at all" and "Completely". Questions refer to the last two weeks.

F2.1(F2.1.1) Do you have enough energy for everyday life?

Not at all	A little	Moderately	Mostly	Completely
1	2	3	4	5

F7.1(F9.1.2) Are you able to accept your bodily appearance?

Not at all	A little	Moderately	Mostly	Completely
1	2	3	4	5

F10.1(F12.1.1)To what extent are you able to carry out your daily activities?

Not at all	A little	Moderately	Mostly	Completely
1	2	3	4	5

F11.1(F13.1.1)How dependent are you on medications?

Not at all	A little	Moderately	Mostly	Completely
1	2	3	4	5

F14.1(F18.1.2)Do you get the kind of support from others that you need?

Not at all	A little	Moderately	Mostly	Completely
1	2	3	4	5

14.2(F18.1.5)To what extent can you count on your friends when you need them?

Not at all	A little	Moderately	Mostly	Completely
1	2	3	4	5

17.2(F21.1.2)To what degree does the quality of your home meet your needs?

Not at all	A little	Moderately	Mostly	Completely
1	2	3	4	5

18.1(F23.1.1)Have you enough money to meet your needs?

Not at all	A little	Moderately	Mostly	Completely
1	2	3	4	5

20.1(F25.1.1)How available to you is the information that you need in your day-to-day life?

Not at all	A little	Moderately	Mostly	Completely
1	2	3	4	5

F20.2(F25.1.2)To what extent do you have opportunities for acquiring the information that you feel you need?

Not at all	A little	Moderately	Mostly	Completely
1	2	3	4	5

F21.1(F26.1.2)To what extent do you have the opportunity for leisure activities?

Not at all	A little	Moderately	Mostly	Completely
1	2	3	4	5

F21.2(F26.1.3)How much are you able to relax and enjoy yourself?

Not at all	A little	Moderately	Mostly	Completely
1	2	3	4	5

F23.1(F28.1.2)To what extent do you have adequate means of transport?

Not at all	A little	Moderately	Mostly	Completely
1	2	3	4	5

ne following questions ask you to say how **satisfied, happy or good** you have felt out various aspects of your life over the last two weeks . For example, about your mily life or the energy that you have. Decide how satisfied or dissatisfied you are th each aspect of your life and circle the number that best fits how you feel about s. Questions refer to the last two weeks.

(G2.1) How satisfied are you with the quality of your life?

Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

(G2.2) In general, how satisfied are you with your life?

Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

(G2.3) How satisfied are you with your health?

Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

(F2.2.1) How satisfied are you with the energy that you have?

Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

(F4.2.2) How satisfied are you with your sleep?

Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

(F7.2.1) How satisfied are you with your ability to learn new information?

ery dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

(F7.2.3) How satisfied are you with your ability to make decisions?

ery dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

F8.2.1) How satisfied are you with yourself?

ry dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

.4(F8.2.2) How satisfied are you with your abilities?

Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

.4(F9.2.3) How satisfied are you with the way your body looks?

Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

0.3(F12.2.3)How satisfied are you with your ability to perform your daily living activities?

Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

3.3(F17.2.3)How satisfied are you with your personal relationships?

Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

.3(F3.2.1) How satisfied are you with your sex life?

Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

.3(F18.2.2)How satisfied are you with the support you get from your family?

Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

4(F18.2.5)How satisfied are you with the support you get from your friends?

Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

4(F19.2.1)How satisfied are you with your ability to provide for or support others?

Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

4(F20.2.3)How satisfied are you with your physical safety and security?

Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

(F21.2.2)How satisfied are you with the conditions of your living place?

ry dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

(F23.2.3)How satisfied are you with your financial situation?

ry dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

F24.2.1)How satisfied are you with your access to health services?

y dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

F24.2.5)How satisfied are you with the social care services?

y dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

F25.2.1)How satisfied are you with your opportunities for acquiring new skills?

y dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

F25.2.2)How satisfied are you with your opportunities to learn new information?

dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

26.2.3)How satisfied are you with the way you spend your spare time?

dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

27.2.1)How satisfied are you with your physical environment (e.g. pollution, climate, noise, attractiveness)?

dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

7.2.3)How satisfied are you with the climate of the place where you live?

dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

F23.3(F28.2.2)How satisfied are you with your transport?

Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

F13.2(F17.2.1)Do you feel happy about your relationship with your family members?

Very unhappy	Unhappy	Neither happy nor unhappy	Happy	Very happy
1	2	3	4	5

G1(G1.1) How would you rate your quality of life?

Very poor	Poor	Neither poor nor good	Good	Very good
1	2	3	4	5

F15.1(F3.1.1) How would you rate your sex life?

Very poor	Poor	Neither poor nor good	Good	Very good
1	2	3	4	5

F3.1(F4.1.1) How well do you sleep?

Very poor	Poor	Neither poor nor good	Good	Very good
1	2	3	4	5

F5.1(F7.1.3) How would you rate your memory?

Very poor	Poor	Neither poor nor good	Good	Very good
1	2	3	4	5

F19.2(F24.1.5)How would you rate the quality of social services available to you?

Very poor	Poor	Neither poor nor good	Good	Very good
1	2	3	4	5

The following questions refer to **how often** you have felt or experienced certain things, for example the support of your family or friends or negative experiences such as feeling unsafe. If you have not experienced these things at all in the last two weeks, circle the number next to the response "never". If you have experienced these things, decide how often and circle the appropriate number. So for example if you have experienced pain all the time in the last two weeks circle the number next to "Always". Questions refer to the last two weeks.

F1.1 (F1.1.1) How often do you suffer (physical) pain?

Never	Seldom	Quite often	Very often	Always
1	2	3	4	5

F4.2 (F6.1.3) Do you generally feel content?

Never	Seldom	Quite often	Very often	Always
1	2	3	4	5

F8.1 (F10.1.2) How often do you have negative feelings, such as blue mood, despair, anxiety, depression?

Never	Seldom	Quite often	Very often	Always
1	2	3	4	5

The following questions refer to any "**work**" that you do. Work here means any major activity that you do. This includes voluntary work, studying full-time, taking care of the home, taking care of children, paid work or unpaid work. So work, as it is used here, means the activities you feel take up a major part of your time and energy. Questions refer to the last two weeks.

F12.1 (F16.1.1)Are you able to work?

Not at all	A little	Moderately	Mostly	Completely
1	2	3	4	5

F12.2 (F16.1.2)Do you feel able to carry out your duties?

Not at all	A little	Moderately	Mostly	Completely
1	2	3	4	5

F12.4(F16.2.1)How satisfied are you with your capacity for work?

Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

F12.3(F16.1.3)How would you rate your ability to work?

Very poor	Poor	Neither poor nor good	Good	Very good
1	2	3	4	5

The next few questions ask about **how well you were able to move around**, in the last two weeks. This refers to your physical ability to move your body in such a way as to allow you to move about and do the things you would like to do, as well as the things that you need to do.

F9.1(F11.1.1) How well are you able to get around?

Very poor	Poor	Neither poor nor good	Good	Very good
1	2	3	4	5

F9.3(F11.2.2) How much do any difficulties in mobility bother you?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

F9.4(F11.2.3) To what extent do any difficulties in movement affect your way of life?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

F9.2(F11.2.1) How satisfied are you with your ability to move around?

Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
1	2	3	4	5

The following few questions are concerned with **your personal beliefs**, and how these affect your quality of life. These questions refer to religion, spirituality and any other beliefs you may hold. Once again these questions refer to the last two weeks.

F24.1(F29.1.1)Do your personal beliefs give meaning to your life?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

F24.2(F29.1.3)To what extent do you feel your life to be meaningful?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

F24.3(F29.2.2)To what extent do your personal beliefs give you the strength to face difficulties?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

F24.4(F29.2.3)To what extent do your personal beliefs help you to understand difficulties in life?

Not at all	A little	A moderate amount	Very much	An extreme amount
1	2	3	4	5

ABOUT YOU

Vhat is your gender?

Male
Female

Vhat is your date of birth?

_____/_____/_____
DAY / MONTH / YEAR

Vhat is highest education you received?

Primary school
Secondary school
University
Post-graduate

hat is your marital status?

Single
Married
Living as married
Separated
Divorced
Widowed

w is your health? (G1.2)**

Very poor	Poor	Neither poor nor good	Good	Very good
1	2	3	4	5

at health problems do you have at the moment? (TICK NEXT TO THOSE THAT APPLY TO YOU)

Heart trouble
High blood pressure
Arthritis or Rheumatism
Cancer
Emphysema or chronic bronchitis
Diabetes
A cataract
Stroke
Broken or fractured bone
Chronic nervous or emotional problems
Chronic foot trouble (bunions, ingrowing toenails)
Rectal growth or rectal bleeding
Parkinson's disease
Other (please describe)

you currently ill?

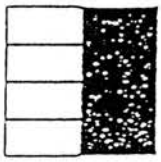
If yes, what is your diagnosis? _____

This question was originally in the body of rated questions in the pilot questionnaire.

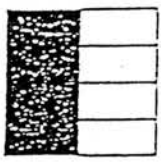
Read each item and place a tick in the box opposite the reply which comes closest to how you have been feeling in the past week.

Don't take too long over your replies: your immediate reaction to each item will probably be more accurate than a long thought-out response.

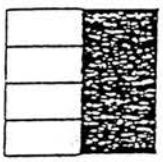
I feel tense or 'wound up':
- Most of the time .....
- A lot of the time .....
- Time to time, Occasionally .....
- Not at all .....



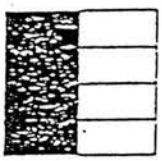
I still enjoy the things I used to enjoy:
- Definitely as much .....
- Not quite so much .....
- Only a little .....
- Hardly at all .....



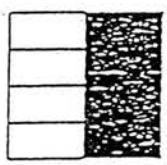
I get a sort of frightened feeling as if something awful is about to happen:
- Very definitely and quite badly .....
- Yes, but not too badly .....
- A little, but it doesn't worry me .....
- Not at all .....



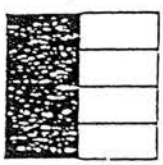
I can laugh and see the funny side of things:
- As much as I always could .....
- Not quite so much now .....
- Definitely not so much now .....
- Not at all .....



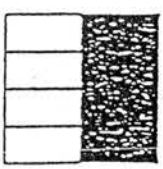
Worrying thoughts go through my mind:
- A great deal of the time .....
- A lot of the time .....
- From time to time but not too often ..
- Only occasionally .....



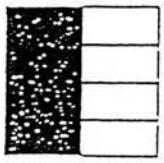
I feel cheerful:
- Not at all .....
- Not often .....
- Sometimes .....
- Most of the time .....



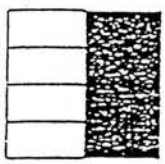
I can sit at ease and feel relaxed:
- Definitely .....
- Usually .....
- Not often .....
- Not at all .....



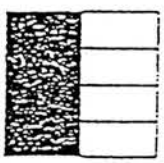
I feel as if I am slowed down:
- Nearly all the time .....
- Very often .....
- Sometimes .....
- Not at all .....



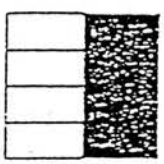
I get a sort of frightened feeling like 'butterflies' in the stomach:
- Not at all .....
- Occasionally .....
- Quite often .....
- Very often .....



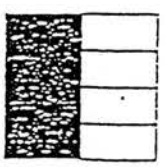
I have lost interest in my appearance:
- Definitely .....
- I don't take so much care as I should.....
- I may not take quite as much care .....
- I take just as much care as ever .....



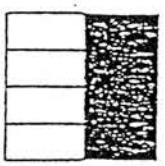
I feel restless as if I have to be on the move:
- Very much indeed .....
- Quite a lot .....
- Not very much .....
- Not at all .....



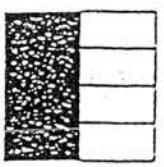
I look forward with enjoyment to things:
- As much as ever I did .....
- Rather less than I used to .....
- Definitely less than I used to .....
- Hardly at all .....



I get sudden feelings of panic:
- Very often indeed .....
- Quite often .....
- Not very often .....
- Not at all .....



I can enjoy a good book or radio or TV programme:
- Often .....
- Sometimes .....
- Not often .....
- Very seldom .....



BENTALL INVENTORY

Please circle the appropriate item to indicate how much you have been bothered by the following during the last month:

Spells of confusion	NOT AT ALL	A LITTLE	SOMEWHAT	QUITE A LOT	VERY MUCH
Thoughts getting mixed up	NOT AT ALL	A LITTLE	SOMEWHAT	QUITE A LOT	VERY MUCH
Poor concentration	NOT AT ALL	A LITTLE	SOMEWHAT	QUITE A LOT	VERY MUCH
Can't easily make decisions	NOT AT ALL	A LITTLE	SOMEWHAT	QUITE A LOT	VERY MUCH
Poor memory for recent events	NOT AT ALL	A LITTLE	SOMEWHAT	QUITE A LOT	VERY MUCH
Can't take things in when speaking to people	NOT AT ALL	A LITTLE	SOMEWHAT	QUITE A LOT	VERY MUCH
Thoughts are slow	NOT AT ALL	A LITTLE	SOMEWHAT	QUITE A LOT	VERY MUCH
Muzzy head	NOT AT ALL	A LITTLE	SOMEWHAT	QUITE A LOT	VERY MUCH
Can't find the right words	NOT AT ALL	A LITTLE	SOMEWHAT	QUITE A LOT	VERY MUCH

APPENDIX 3

Prevalence, Incidence, and Clinical Characteristics of Hepatitis G Virus/GB Virus C Infection in Scottish Blood Donors

C. S. Blair, F. Davidson, C. Lycett, D. M. McDonald, G. H. Haydon, P. L. Yap, P. C. Hayes, P. Simmonds, and J. Gillon

Departments of Medicine and of Medical Microbiology, University of Edinburgh, Royal Infirmary of Edinburgh, and Southeast Scotland Blood Transfusion Service, Edinburgh, United Kingdom

The prevalence, incidence, clinical features, and natural history of hepatitis G virus (HGV) or GB virus C (GBV-C) were investigated in a non-remunerated blood donor population to determine its clinical significance and its impact on blood safety. Of 1020 regular blood donors, 23 (2.25%) were positive for plasma HGV/GBV-C RNA. Alanine aminotransferase levels were lower than in uninfected donors (median, 20 IU/mL; 32 IU/mL in controls; $P = .015$). Clinical examination produced no other evidence for hepatitis or for shared nonhepatic diseases. Fifteen of 17 donors excreted HGV/GBV-C in saliva (mean level, 8×10^3 copies of RNA/mL). Testing of previous donations indicated an incidence of 170–200 new infections with HGV/GBV-C per 100,000 donor-years. The absence of further clinicopathologic data and the limitations of current polymerase chain reaction–based methods for screening suggests that it is neither necessary nor practical to commence screening.

Hepatitis C virus (HCV) has been identified as the principal cause of posttransfusion hepatitis, while the development of serologic tests for blood donor screening has led to a dramatic fall in its incidence. However, in 10% of occurrences of posttransfusion hepatitis and 20% of community-acquired hepatitis, no etiologic agent has been identified. GB virus C (GBV-C) [1] or hepatitis G virus (HGV) [2] has been proposed as the etiologic agent of these residual cases. In this study, we aimed to determine the prevalence of HGV/GBV-C in a large sample of the Scottish donor population and the natural history of this population's infection with the virus and to assess the clinical significance and possible risk factors for transmission of infection in HGV/GBV-C–positive donors.

Subjects and Methods

Subjects. From October 1996 until December 1996, 1020 blood samples were collected from volunteer, consecutive, regular blood donors attending the Edinburgh and Southeast Scotland Blood Transfusion Service center. Consent to participate in the screening program was obtained from 1020 (97%) of 1052 donors interviewed. All subjects were seronegative for human immunodeficiency virus (HIV), hepatitis B virus (HBV), and HCV. All HGV/GBV-C–positive donors were contacted and invited to attend for counseling and further investigation. At their initial consultation, repeat sampling for plasma HGV/GBV-C RNA and alanine aminotransferase (ALT) measurement was undertaken, and a history of risk behavior was sought. In addition, a sample of saliva was collected for testing for HGV/GBV-C RNA. All positive donors were referred to a single hepatologist for clinical evaluation.

Detection of HGV/GBV-C RNA and antibody in plasma. Detection and quantitation of HGV/GBV-C was done as previously described [3] on RNA extracted from 100 μL of EDTA–anticoagulated plasma or citrated plasma from blood donors that was combined into pools of 10. Positive pools were subdivided into component donations and retested. Initial screening identified 23 positive pools, which led to the identification of one positive component donation in each. RNA was also extracted from 100-μL volumes of saliva samples as described for plasma samples. IgG antibodies to GBV-C/HGV E2 antigen were detected by assay (PLATE Anti-HGenv; Boehringer Mannheim, Mannheim, Germany).

Results

Prevalence of HGV/GBV-C in blood donors. Twenty-three (2.25%) of 1020 healthy blood donors were positive for HGV/GBV-C RNA in plasma (11 men [mean age, 37.9 years] and 12 women [mean age, 33.3 years]; table 1). Nineteen returned for further investigation ~10 weeks after donation, and 18 remained HGV/GBV-C RNA–positive. HGV/GBV-C was detected in 13 of 17 saliva samples from HGV/GBV-C–viremic donors collected at that time. Levels in saliva were ~3 log lower than found in plasma (geometric means, 7.8×10^3 and 7.4×10^6 copies of HGV/GBV-C RNA/mL, respectively; table 1), but levels showed a weak correlation ($R = .442$, $P = .05$ [Spearman's rank correlation test]). The donor who had become

Received 9 April 1998; revised 15 July 1998.

Presented in part: British Blood Transfusion Service Meeting, Warwick, UK, September 1997.

Consent for HGV/GBV-C testing, recall, and examination was obtained from all blood donors who participated in the study. The study was approved by the Lothian Research Ethics Committee.

Grant support: Scottish National Blood Transfusion Service.

Reprints or correspondence: Dr. Carol Blair, Department of Medicine (RIE), University of Edinburgh, Royal Infirmary, Lauriston Place, Edinburgh, EH3 9YW, United Kingdom.

The Journal of Infectious Diseases 1998; 178:1779–82

0022-1899/98/7806-0032$02.00

Table 1. Epidemiologic and clinical characteristics of HGV/GBV-C–infected blood donors.

Donor[a]	Age	Sex	ALT, IU/mL	Previous donations	Plasma copies/mL[b]	Saliva copies/mL[b]	Duration of Infection,[c] years
p12	20	M	25	6	5×10^6	<100	1
p11	21	F	18	10	5×10^8	5×10^4	>2
p15	25	F	18	12	5×10^7	5×10^3	1
p16	26	F	20	17	5×10^8	5×10^4	2
p8	27	F	13	6	5×10^7	5×10^4	6
p17	27	M		14			>8
p6	28	F	18	19	<100	4×10^3	5
p9	28	F	15	9	5×10^6	5×10^4	6
p5	29	M	38	6	5×10^7	5×10^5	10
p3	30	M	47	24	5×10^5	4×10^3	2
p2	32	F		7			9
p14	32	M	33	7			>5
p4	36	F		6			>11
p18	39	M	30	28	5×10^6	5×10^3	6
p19	39	F	27	19	5×10^8	5×10^3	8
p13	40	F	15	12	5×10^7		4
p22	44	M		42			7
p7	45	F	19	11	5×10^7	5×10^3	>10
p1	47	M	20	24	5×10^6	5×10^5	7
p20	48	M	34	47	5×10^7	5×10^3	9
p10	50	M	8	8	5×10^7	5×10^4	3
p21	51	M	43	56	3×10^4	<100	9
p23	53	F	31	47	5×10^6	5×10^5	9

NOTE. ALT, alanine aminotransferase.

[a] Donors are ranked by age.

[b] HGV/GBV-C load in plasma and saliva from donor on recall.

[c] Approximate duration of HGV/GBV-C infection; time of infection is calculated as midpoint between last polymerase chain reaction–negative and first polymerase chain reaction–positive donation; in most cases these are minimum estimates, as donors have been followed prospectively for at longest 1 year (figure 1).

polymerase chain reaction (PCR)–negative in plasma produced a saliva sample that was PCR-positive (4000 copies of HGV/GBV-C RNA/mL).

One year after the original study, 11 of 14 donors who attended for follow-up remained HGV/GBV-C RNA–positive. The 3 donors who became PCR-negative showed the lowest virus loads in plasma at time of original donation (p3, 5×10^5; p18, 5×10^6; and p21, 3×10^4 copies of HGV/GBV-C RNA/mL; $P = .017$ [Kruskal-Wallis test]). The sample from p3 was reactive in the Boehringer anti-E2 ELISA (optical density [OD] of 1.6), but those from p18 and p21 were negative (ODs of 0.036 and 0.073, respectively).

Clinical assessment of HGV/GBV-C–infected blood donors. Two donors (p13, p20) of the 18 donors recalled for clinical assessment had a history of blood transfusion. Several donors had undergone ear piercing or tattoos but in establishments where virus transmission would not be considered likely. None had a history of intravenous drug use or sexual contact with a person at high risk of parenteral virus infection.

Fourteen of 23 HGV/GBV-C–positive donors were clinically examined. None had any history of jaundice or symptoms or stigmata of chronic liver disease or hepatomegaly. Five of 18 donors reported various musculoskeletal symptoms, although not of a consistent pattern and of uncertain relationship to HGV/GBV-C infection. Liver function tests were carried out for the 19 donors returning after original donation (table 1). Two donors showed a minimal elevation in ALT level (47 and 43 IU/mL [normal range, 10–40 IU/mL]). The median ALT level among HGV/GBV-C–infected donors was 20 IU/mL, lower than the median of 32 for a control group of 100 HGV/GBV-C–uninfected blood donors ($P = .015$) and a median of 61 for 91 HCV-infected donors ($P < .001$) [4]. All other liver function tests (bilirubin, alkaline phosphatase, and γ glutamyl transferase) were similarly within the normal range. All measurements in a full blood count were in the normal range for the 19 donors, apart from mild lymphopenia in 5.

Donations from 2 partners of HGV/GBV-C–infected donors who donated at the time of the original study were HGV/GBV-C PCR-negative. Three partners who attended the counseling and follow-up session were negative for HGV/GBV-C RNA in plasma and saliva and negative for antibody to HGV/GBV-C.

Incidence of HGV/GBV-C infection in blood donors. Archived samples from previous blood donations of all 23 HGV/GBV-C–infected donors were tested by PCR (figure 1). All samples from 6 donors (collected during 1986–1994) were PCR-positive, while the earliest samples from the remaining 17 (1983–1995) were PCR-negative. Two donors (p10 and p19) became PCR-positive in 1994 and 1992, respectively, but over the next 1–2 years became transiently PCR-negative and negative for antibody to E2, before becoming viremic again at the end of 1996. A total of 17 blood donors became infected with HGV/GBV-C over a mean observation period of 9.7 years. The number of susceptible blood donors investigated was 1046 (excluding the persistently viremic donors during the observation period), allowing an approximate calculation of the incidence of HGV/GBV-C infection of 0.17% per year.

One explanation for the large number of HGV/GBV-C–negative samples collected before 1990 is that HGV/GBV-C RNA sequences had degraded on long-term storage. We recalculated the incidence of HGV/GBV-C infection using shorter time intervals. When we used only the results from 1990 onwards, a total of 13 blood donors became infected over a mean interval of 6.2 years (incidence, 0.20% per year). Similarly, 8 became positive from 1992 onwards (mean observation period, 4.5 years; incidence, 0.17% per year). The similarity in these estimates suggests that degradation of HGV/GBV-C RNA during storage did not affect the calculation of incidence.

Discussion

In this study, we have demonstrated that 23 (2.25%) of 1020 healthy non-remunerated blood donors had plasma samples positive for HGV/GBV-C RNA by reverse transcription–PCR. This frequency lies within the range reported for comparable non-remunerated blood donor populations in other countries, such as Germany (1.3%–1.9%), France (4.2%), the United

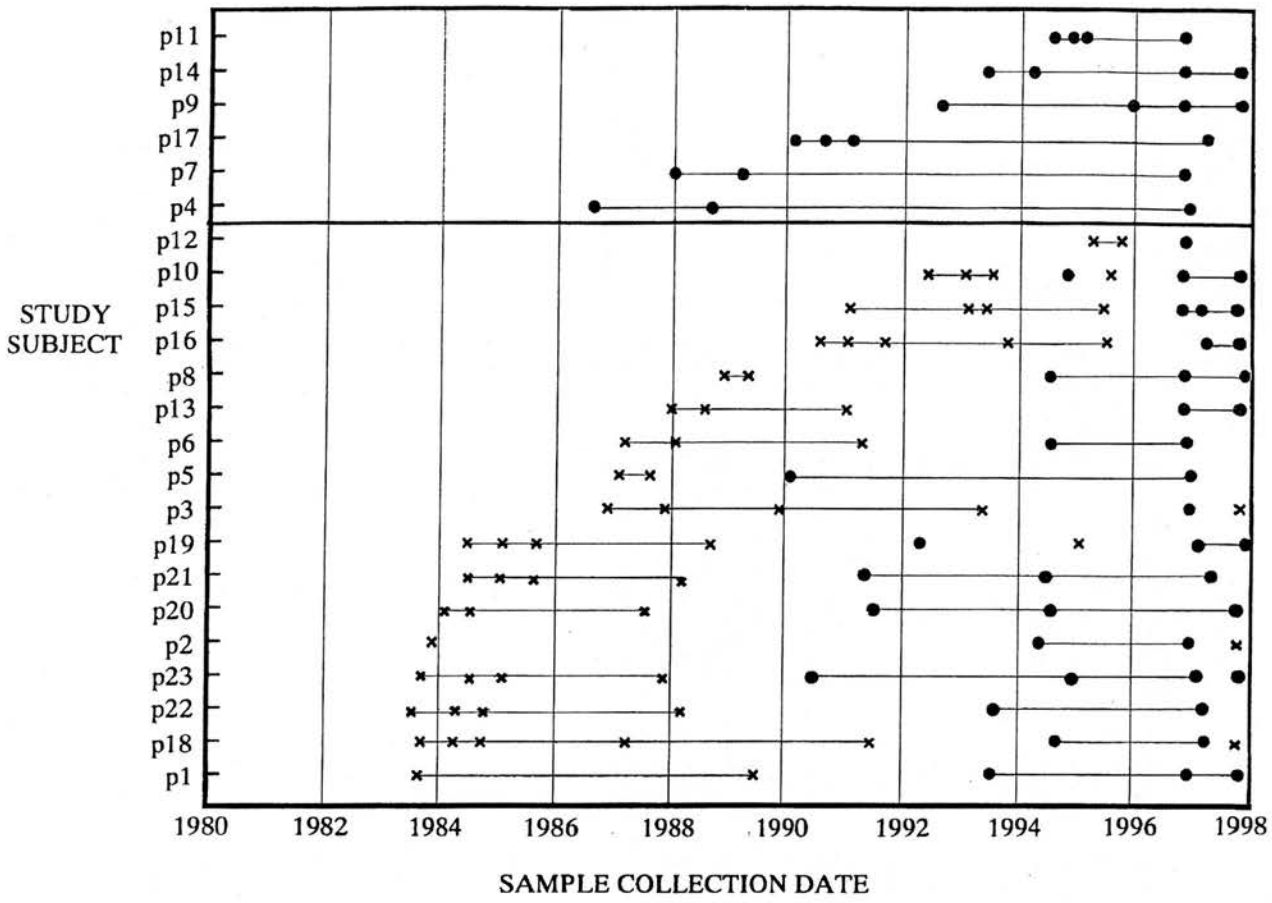


Figure 1. Retrospective testing for HGV/GBV-C RNA of archived blood donations from 23 blood donors identified as HGV/GBV-C–infected in November–December 1996. 6 donors above horizontal line are those with persistent infection throughout observation period. ●, HGV/GBV-C polymerase chain reaction–positive; ×, polymerase chain reaction–negative.

States (1.7%), Australia (4%), and Japan (0.5%–1.2%) [2, 5–8]. The male-to-female ratio of HGV/GBV-C–infected donors was 11:12, different from the 3:1 ratio observed among blood donors infected with HCV in Scotland or elsewhere. The high prevalence of HGV/GBV-C infection and the absence of significant disclosed parenteral risk factors for infection in the HGV/GBV-C–infected donors strongly suggests other routes of transmission.

Among other proposed routes of transmission of HGV/GBV-C, evidence for transmission by sexual contact includes the finding of high prevalences of infection in persons with sexually transmitted disease or with other evidence of sexual exposure with multiple partners [9]. Against this hypothesis is the absence of detectable transmission of HGV/GBV-C (either by PCR or serology) to the sex partners of 5 infected donors. Mother-to-child transmission has been documented by the frequent detection of HGV/GBV-C viremia (20%–70%) in children born of infected mothers [10]. However, the observation of de novo infection in at least 17 of the 23 HGV/GBV-C–infected blood donors in the current study indicates that the acquisition of infection occurred predominantly in adulthood.

The frequent finding of HGV/GBV-C–positive samples among archived donations from the infected donors indicates that infection may persist for several years (at least 10 years in p4). The results of this retrospective study are consistent with the finding of persistent infection of at least 2 years among 4 of 5 Australian blood donors [7] and the documented prolonged infection in varying proportions of persons infected with HGV/GBV-C through blood transfusion or treatment with blood products [11, 12].

A surprising finding that arose from the retrospective study was the relatively high incidence of HGV/GBV-C infection. The calculated rate (170–200 infections per 100,000 person-years) contrasts strongly with rates of acquisition of HCV (1.8–10 per 100,000), HIV (1.3–4.0 per 100,000), and HBV (1.7–4.0 per 100,000) in similar non-remunerated blood donor populations in Europe, the United States, Japan, and Australia.

If, in the future, donations were to be screened for HGV/GBV-C RNA by PCR, the number of new infections detected would place severe constraints on the size of the pool used. For example, even if all persistently infected donors were excluded, new infections with HGV/GBV-C over a mean donation interval of 6 months would lead to contamination of 50% of pools containing 500 component donations and 10% of pools containing 100 components.

The asymptomatic nature of HGV/GBV-C was directly demonstrated by our clinical examination of the infected donors. Two donors showed minimal elevation of ALT levels, but the mean level (20 IU/mL) and all other liver function tests were in the normal range. No subject had a previous history of acute hepatitis or jaundice. No donor had any abnormality on clinical examination, other than arthralgia in 5 persons. However, there was no consistent pattern of joint involvement, and larger studies would be required to make a more substantive link.

These findings are consistent with the absence of clinically or biochemically apparent liver disease in those infected by blood transfusion [13–15]. For example, of 79 with posttransfusion hepatitis, only 3 were infected with HGV/GBV-C alone [14], all had mild, asymptomatic hepatitis, and the absence of any correlation between ALT elevation and detection of HGV/GBV-C by PCR suggests other causes for the observed hepatitis. A difficulty encountered in investigating the HGV/GBV-C–infected donors in the current study arose from the current lack of information about the tropism and likely disease associations of HGV/GBV-C in vivo. Although HGV/GBV-C RNA can be detected in the liver, there is little evidence for its replication there. Thirteen of 17 HGV/GBV-C–positive donors showed relatively high levels of HGV/GBV-C RNA in saliva and a mean ratio to levels detected in plasma of ~1:1000. The frequency of detection and ratio to levels detected in plasma contrasts with that documented for HCV and suggests that replication of HGV/GBV-C in the respiratory or gastrointestinal tracts may occur. None of them had any history of respiratory symptoms or disease, although its secretion into saliva may represent a route of transmission, as documented for human cytomegalovirus and other herpesviruses.

In conclusion, this study demonstrates that HGV/GBV-C infection is common among our donor population but is not associated with significant hepatic disease or symptoms. We have not been able to identify a risk factor for infection in the majority of HGV-positive donors. HGV/GBV-C RNA was present in the plasma in the majority of subjects for several years and was likely to have been transfused in a large number of blood components. These findings will enable recipients of HGV/GBV-C–positive blood to be identified and the clinical sequelae investigated. In the interim, in the absence of clinicopathologic data suggesting significant morbidity in HGV/GBV-C–infected donors, blood donors should not be screened for HGV/GBV-C.

Acknowledgment

We acknowledge the assistance of the staff of the Edinburgh Regional Transfusion Centre for collection of donor samples.

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